NATURE

No. 4803 SATURDAY, NOVEMBER 18, 1961 Vol. 192

CONTENTS

	Page
EDUCATION FOR LEADERSHIP	587
SURVEY OF ANTIBIOTIC LITERATURE. By Dr. B. K. Kelly	589
TRAITÉ DE ZOOLOGIE. By Prof. C. H. O'Donoghue	590
POLYMER CHEMISTRY. By Dr. I. C. McNeill	591
QUANTITATIVE BIOLOGY. By Sir Hans Krebs, F.R.S.	591
A NEW STANDARD WORK ON COCOA	592
LONDON LIBRARIES. By R. Brightman	592
A MICROBIOLOGICAL EXAMINATION OF SOME CARBONACEOUS CHONDRITES. By Dr. George Claus and Prof. Bartholomew Nagy	594
BRADYKININ. By Dr. G. P. Lewis	596
FOOD PROCESSING IN RELATION TO HEALTH. By Miss D. F. Hollingsworth, O.B.E.	599
SPEECH	601
OBITUARIES :	
PROF. H. V. A. BRISCOE. By Prof. G. Wilkinson	604
PROF. CARL HERMANN. By Dame Kathleen Lonsdale, D.B.E., F.R.S.	604
PROF. E. TAYLOR JONES. By Dr. S. C. Curran, F.R.S.	605
DR. MADELINE KERR. By Dr. D. Chapman	605
NEWS AND VIEWS	606
THE UNIVERSITY OF OXFORD EXPEDITION TO PERU, 1961. By R. C. Herrera	610
ELECTRON MICROSCOPY IN THE UNITED STATES. By A. R. Taylor	611
THERMIONIC CONVERSION OF HEAT TO ELECTRICITY. By R. V. Harrowell	611
RESEARCH IN THE LEATHER INDUSTRY. By R. G. Mitton	612
INTERNATIONAL UNION OF FOREST RESEARCH ORGANIZATIONS. By F. C. Ford-Robertson	614
NUTRITION AND POULTRY. By M. E. Coates	615
THE EUROPEAN NUCLEAR ENERGY AGENCY	616
UNIVERSITY FINANCE IN BRITAIN: 1959–60	617
HEALTH SERVICES IN SMALL FACTORIES	618
UNIVERSITY OF SUSSEX	619
METABOLISM OF THE HUMAN RED BLOOD CELL	619
STUDENT MENTAL HEALTH. By Dr. Clifford Allen	620
TRAWL FISHING IN THE TROPICAL ATLANTIC. By R. H. Lowe (McConnell) and Alan R. Longhurst ; Alan R. Longhurst	620
A NEW APPLICATION OF THE FARADAY MAGNETO-OPTICAL EFFECT FOR DIAGNOSTIC MEASUREMENTS OF TRANSIENT MAGNETIC FIELDS. By R. D. Medford, W. H. W. Fletcher, J. D. Herbert and A. L. T. Powell	622
SOME PHYSICO-CHEMICAL PROPERTIES OF PARACRYSTALLINE SPHERULITES OF BIOLOGICAL ORIGIN. By Dr. G. T. Stewart	624
KINETICS OF REACTION OF THE DECOMPOSITION OF LEAD AZIDE. By Dr. M. Stammler, J. E. Abel and Dr. R. C. Ling	626
PROBABILITY DISTRIBUTION ANALYSER PROGRAMMED FOR NEUROPHYSIOLOGICAL RESEARCH. By Dr. W. R. Levick, Prof. P. O. Bishop, W. O. Williams and Prof. D. G. Lampard	629
HÆMIN-PROTEIN BINDING IN PEROXIDASE AND METHÆMALBUMIN. By Dr. A. C. Maehly	630
TRANSFERRIN DIFFERENCES IN CHIMPANZEE SERA. By Prof. J. Buettner-Janusch	632
MECHANISM OF STREPTOMYCIN ACTION ON BACTERIA : A UNITARY HYPOTHESIS. By Dr. Charles R. Spotts and Prof. R. Y. Stanier	633
TEMPERATURE CHARACTERISTICS OF BACTERIAL VARIATION IN LEGUME SYMBIOSIS. By Dr. J. S. Pate	637
CRYSTALLIZATION OF CYTOCHROME b_2 FREE OF THE DEOXYRIBONUCLEIC ACID COMPONENT. By Prof. R. K. Morton and Kathryn Shepley	639
POSSIBLE CAUSES FOR PRODUCTION OF ISOLEUCINE BY *Streptomyces rimosus*. By Dr. I. Horváth, I. Gadó and A. Szentirmai	641
A SUBCELLULAR COMPONENT OF *Corynebacterium diphtheriae* HAVING THE CAPACITY TO BIND ANTI-TOXIN. By Prof. M. Yoneda and M. Matsuda	642

(Continued overleaf)

UNICAM

This is the SP.700 Spectrophotometer. One of the most advanced analytical instruments of its kind. Precise—versatile—reliable—made for analysis through a spectral range of Ultraviolet, Visible and Near Infrared. The SP.700 records a detail of the composition of unknown mixtures—detects minute traces of the most elusive elements—completes in minutes a task which would take hours or weeks by other methods. Used by scientists in the great University and Industrial Laboratories of the world. Made by Unicam Instruments Ltd., York Street, Cambridge.

SP.700 Recording Spectrophotometer

Photograph reproduced by permission of Parke Davis & Co. Ltd

UNICAM INSTRUMENTS LIMITED YOR STREET CAMBRIDGE

LETTERS TO THE EDITORS

Page

GEOCHEMISTRY

Uranium in the Sikhote-Alin Meteorite and its Relation to the Lead Method of Age Determination.—Edward L. Fireman and David E. Fisher 644

GEOPHYSICS

Palæomagnetism of Hawaiian Lava Flows.—Richard R. Doell and Allan Cox . . . 645

PHYSICS

Emission of Ultra-Violet and Visible Radiation from Metals by the Impact of High-Energy Hydrogen Ions.—Prof. Rafi Mohammed Chaudhri and Mustafa Yar Khan 646

High-Speed Direct-Recording System for Electron Diffraction.—Dr. C. W. B. Grigson . . 647

Isotopic Composition of Atmospheric Hydrogen and Methane.—A. E. Bainbridge, Prof. H. E. Suess and Irving Friedman; K. F. Bishop, B. T. Taylor and A. E. J. Eggleton . . . 648

ENGINEERING

A Novel Electrostatic Machine: the Corona Motor.—J. D. N. Van Wyk and G. J. Kühn . 649

Rectifying Action of Non-uniform Fields in Dielectrics.—Dr. K. C. Kao 650

CHEMISTRY

Experimental Detection of Auto-Ionizing Transitions in Krypton by Electron Impact.—John F. Burns 651

Surface Properties of Silver Iodide.—Prof. A. C. Zettlemoyer, Noubar Tcheurekdjian and J. J. Chessick 653

Production of 'Active' Magnesium Oxide by heating in Vacuum.—R. Valverde and J. P. Sáenz 653

Catalytic Decomposition of Ethylene on Nickel.—Douglas W. McKee 654

Role of Keto-Enol Tautomerism in the Polarographical Reduction of Some Carbonyl Compounds.—Dr. P. Zuman and Dr. J. Michl . 655

BIOCHEMISTRY

Modification of the Properties of Flour Protein by Thiolated Gelatin.—E. E. McDermott and J. Pace 657

Separation of Low Molecular Weight Peptides from Amino-Acids on 'DEAE-Sephadex'.—P. R. Carnegie 658

Demonstration and Some Properties of Peroxidase in Pig Thyroid Gland.—Toichiro Hosoya and Nobuo Ui 659

Transaminase Activity in Arterial Tissue of the Rabbit.—Dhurjati Prasad Chattopadhyay . 660

Interaction of ^{14}C-Œstrone with Tryptophan in the Presence of Peroxidase.—Dr. P. H. Jellinck and Louise Irwin 660

Auxin Activity in Ether-insoluble Fractions of Extracts of Rhubarb Petioles.—B. E. S. Gunning 661

PHYSIOLOGY

Peroxide-like Substance in Alarmed Mice.—V. J. Horgan and J. St. L. Philpot . . . 662

Evidence for Independence of Voltage of the Membrane Conductance of Rabbit Ventricular Fibres.—Dr. Edward A. Johnson and J. Tille . 663

Effect of L-Glutamic Acid on the Weight of Embryonic Chick Tibiotarsi cultivated *in vitro*.—Dr. J. D. Biggers 664

Effects of Intraventricularly Injected Anti-N. Caudatus Antibody on the Electrical Activity of the Cat Brain.—Dr. Lj. Mihailović and Dr. B. D. Janković 665

Page

Effect of Colchicine on Rat Liver Xanthine Oxidase.—Dr. Ottilia R. Affonso, Emilio Mitidieri and Gilberto G. Villela 666

Effect of Hydrocortisone on Uptake of Iron-59 in Incubated Rat Red Cells *in vitro*.—Peggy Clark 667

Action of Methyl Hydroxybenzoate on Nervous Conduction.—Dr. P. W. Nathan and T. A. Sears 668

HÆMATOLOGY

Alkali-resistant Hæmoglobins in Prosimian Primates.—John Buettner-Janusch and Joseph B. Twichell 669

PATHOLOGY

Interaction of the Carcinogenic *N*-Methyl-Nitrosourethane with Sulphydryl Groups.—Dr. R. Schoental 670

Antagonism between Antibacterial Drugs.—A. Manten and M. J. Wisse 671

New Renal Tubular Amino-Acid Transport System and a New Hereditary Disorder of Amino-Acid Metabolism.—Dr. C. R. Scriver, I. A. Schafer and M. L. Efron 672

BIOLOGY

Serological Relationships between the Nine-spined and the Three-spined Stickleback.—Tadeusz Penczak 673

Biotin, a Growth Requirement for Four Soil Amœbæ.—R. N. Band 674

Effect of Ribonuclease on Shell Regeneration in *Ariophanta* sp.—K. K. Nair and P. T. Muthe . 674

Action of a Detergent (Dodecylbenzenesulphonate) on the Gills of the Trout.—O. J. Schmid and H. Mann 675

The Species of the Teleostean Genus *Lebetus*.—P. J. Miller 675

Manner of expressing Rate of Ribonucleic Acid Biosynthesis.—D. G. Fraenkel and F. C. Neidhardt 677

Effect of Temperature and Potassium Gibberellate on Phases of Growth of Algerian Ivy.—J. R. Goodin and Prof. V. T. Stoutemyer . . 677

Effect of Gibberellic Acid, Kinetin and Other Substances on Seed Dormancy.—B. Frankland 678

Bioassay of Gibberellic Acid using Excised Embryos of *Avena fatua* L.—Prof. J. M. Naylor and G. M. Simpson 679

ENTOMOLOGY

Occurrence of a Hyperglycæmic Factor in the Corpus Cardiacum of an Insect.—J. E. Steele . 680

Inhibited Oviposition by Females of *Gryllus assimilis* (F.), induced by Radioactive Males, using L-Methionine-Methyl-^{14}C.—A. A. Abdel-Malek and D. K. McE. Kevan 681

MICROBIOLOGY

Survival of *Rhizobium meliloti* in Soil Culture.—H. L. Jensen 682

Inhibition of Lysozyme by 'Suramin'.—Dr. Iwo Lominski and Samuel Gray 683

Dihydroxyacetone Production in *Acetomonas*.—Dr. J. G. Carr and J. L. Shimwell . . 683

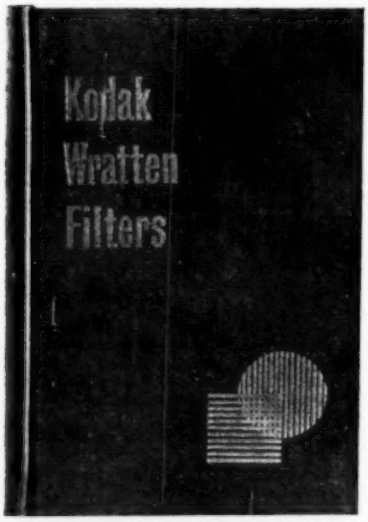
CYTOLOGY

Chromosome Preparations obtained directly from Peripheral Blood.—M. A. Kinlough and Prof. H. N. Robson 684

Surface Granulation in Myeloid Leucocytes.—Dr. A. D. McCutcheon 684

ARCHÆOLOGY

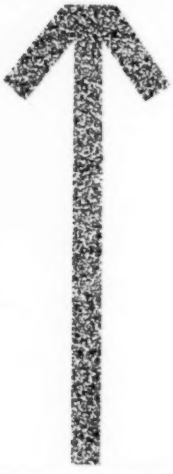
Absolute Age of Pleistocene and Holocene Deposits in the Haua Fteah.—Dr. C. B. M. McBurney . 685



Kodak

Kodak Limited, Industrial Sales Division,
Kodak House, Kingsway,
London W.C.2.

Kodak Wratten Filters

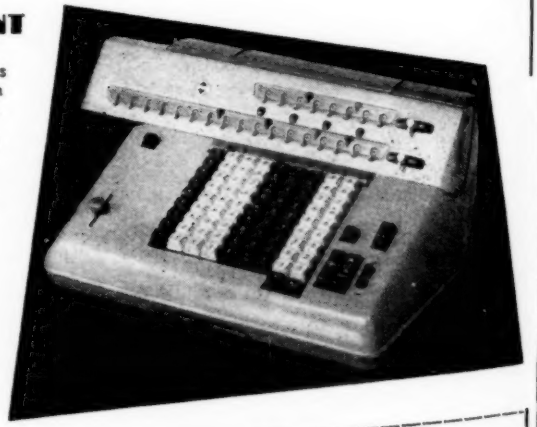


The second edition of the book 'Kodak Wratten Filters', is now available. A work of reference for photographers, industrial and scientific laboratory technicians and others who require information concerning the selective absorption of light, it gives full information on all Wratten and other filters manufactured by Kodak Ltd.

Attractively presented for easy reference, 'Kodak Wratten Filters' includes sections on filters for colour photography, black and white photography, graphic arts use, and technical and scientific use. Also included are chromaticity diagrams, spectro photometric absorption curves and a density/per cent transmission table.

'Kodak Wratten Filters' costs 12/6d and is obtainable from Kodak Limited, Industrial Sales Division.

HIGH PERFORMANCE in the MEDIUM PRICE RANGE is the VA17A ACHIEVEMENT



1. The VA17A Automatic Memory* Calculator retains any factor **during operation** for later use as a constant divisor, dividend, multiplier etc.
2. Extreme simplicity of control—the VA17A can be 'programmed' if work has to be delegated.
3. Fully automatic in **all** operations including negative multiplication and division, zero skipping and automatic squaring.
4. Direct addition in **multiplier** register enabling immediate multiplication of totals—or two totals simultaneously.
5. Direct calculation of intermediate answers without re-setting—a speedy solution to difficult problems.
6. Correct decimal positioning, full tens transmission and so much more than you expect at £285.

Badenia
CALCULATORS LIMITED
LION HOUSE, 72 RED LION ST., W.C.1. HOL 7744

Please send me details of:
- ☐ VA 17 CALCULATORS
- ☐ DIA-PLAN WALL CHART
- ☐ ROL-A-CHART WALL CHART
- ☐ NORWEGIAN "MINIATURE OFFICE" in a cabinet

Name .....
Firm .....
Address .....

EDUCATION FOR LEADERSHIP

IN his presidential review to the Rockefeller Foundation for 1960*, Mr. G. J. Harrar, who succeeded Mr. Deak Rusk as president on July 1, 1961, pays particular attention to the deep interest which the Foundation has taken from the first in the welfare of people in the less-developed countries. Originally this was manifested through the fundamental studies and programmes in public health which its International Health Division undertook in many parts of the world. Now it is equally manifest in the agricultural programme, which, beginning in Mexico, covers co-operative projects in Colombia, Chile, India and the Philippines; and the Foundation's researches on arthropod-borne virus diseases has at various times involved co-operative field stations in six different countries. Supplementing these programmes and partly in support of them is the increased emphasis on training individuals from many countries for leadership in national and international development; and training is now a major sector of the Foundation's whole programme and is strongly oriented toward the less-developed countries. Indeed, taking into account the increasing proportion of the Foundation's grants which seek to strengthen educational and research institutions overseas, education for leadership, particularly in the less-developed countries, could fairly be described as the dominant theme in the President's review; and it is presented with all the lucidity, cogency and balance which have marked the reviews of his distinguished predecessors.

Mr. Harrar notes first that the Foundation's fellowships are awarded to strengthen institutional faculties by enriching the experience of investigators and teachers, as well as for training younger individuals who plan careers in teaching and research. Again, its travel awards have enabled many responsible leaders in all aspects of national planning to observe principles and practices in use elsewhere, and a considerable number of American scholars and scientists have worked for extended periods with colleagues in overseas institutions. Further, individuals who have been associated with Foundation operating programmes or who have received training awards have been given assistance on their return to their parent institution to make them professionally more effective and to strengthen their departments and institutions. Many grants, too, are made each year to institutions, departments and projects in the United States and abroad which seem of promise, the objective being to increase knowledge in its application for the benefit of mankind.

In many countries the most urgent needs are for a steady rise in standards of living and for the broadening of scholastic, intellectual and creative opportunities; but in some, early priority must be given to improvements in sanitation, public health and medical care, and to increased food production as the basis of an adequate diet. Until these requirements are satisfied, Mr. Harrar points out, real progress in housing, transport, communications and other technologies is impossible: scholarly activities and the creative arts are unlikely to flourish among sick or under-nourished people. The need for more qualified persons to accept major responsibilities in government, education, science, technology, and economic and social development is manifest everywhere. Innate ability is not lacking, but opportunities for its full development are often grossly inadequate, and accordingly the most important single element in the progress of the less-developed or emerging States is leadership. Both the quality of training and the number trained are important, and these Mr. Harrar fully recognizes are primarily a national responsibility; philanthropic and other foreign agencies can only help at critical points, particularly at the more advanced levels. Although most future leaders and investigators must necessarily receive their basic training at national institutions, and can only be sent overseas for training on a selective basis, it is for this reason that bodies like the Rockefeller Foundation are especially concerned with training for leadership.

The urgent and growing demands being made on scientific and technological man-power and on professional man-power make it the more important that such resources should be used with skill and perception. Here Mr. Harrar suggests that private foundations operating overseas have a unique opportunity to assist in creating patterns by which the limited numbers of highly trained individuals in less-developed countries can contribute most effectively to research and to the training of younger people. If these leaders are properly situated, supported and encouraged, they can create islands of excellence which, with increasing numbers, may grow and coalesce into a firm base for genuine progress. In view of the many opportunities for investment in excellence in the United States, the Rockefeller Foundation is increasingly selecting those which may ultimately pay substantial dividends both there and abroad; a significant proportion of the Foundation's grants to educational institutions in the United States are now in recognition of their part in training people from the less-advanced countries, and to assist the extension of various phases of institutional programmes in the United States into critical areas abroad.

* The President's Review from the Rockefeller Foundation Annual Report, 1960. Pp. 96. (Rockefeller Foundation, 111 West 50th Street, New York, 1961.)

XUM

In this belief that the greatest challenge to private foundations is their work for the benefit of mankind, Mr. Harrar discusses further the importance of educational developments in the less-developed countries and the contribution of the Foundation's scheme of fellowships and training grants for study abroad. These have been used to assist particularly promising or enterprising individuals, with the object of encouraging the development of growing points of excellence. The Foundation's interest in programmes for the control of specific diseases has almost entirely given place to this type of development. There is, however, one exception, namely, the virtually world-wide problem of malnutrition, and for the past twenty years the Foundation has been attacking this problem at what might be regarded as its source—inadequately developed agricultural practices. Attention has also been given to the adaptation of available food supply to biological needs, and three grants during the current year—one to Harvard University for a co-operative project with the Medical School in Cali, Colombia, one to the National Institute of Nutrition in Mexico, and one to the Institute of Nutrition of Central America and Panama in Guatemala—are cited as contributions in the Foundation's continuing programme for improving theory and practice in nutrition.

The problem of food supply and nutrition is, however, closely linked with that of population and its growth, and here for thirty years the Foundation has sought opportunities to advance knowledge of all aspects of the reproductive process, while latterly the psychological implications of the reproductive process and sociological study of the pattern of population growth have received attention. This latter aspect has received particular attention from the Population Council, which has done a great deal to increase world knowledge of the population problem, and last year arranged with the Johns Hopkins School of Public Health that the School should add to its staff experts in various phases of the population question. Some of their time will be spent at Johns Hopkins studying matters of basic theory and practical application; but an appropriate portion of their time will be available to the Population Council for field-work in co-operation with government health services responsible for planning more orderly changes in the size of population.

The Rockefeller Foundation has participated in initiating this new arrangement, which should increase the number of competent investigators of the difficult problem of how to change public attitude and practice in this field. For the past five years, too, the Foundation has been exploring possible ways of increasing the supply of well-trained diplomats for the execution of foreign policy and the effective representation of the interests of the emerging countries' interests abroad. The critical importance of this factor has been repeatedly demonstrated by the events of the past year, but in this field assistance in training well-qualified leaders is a far more complex problem and directly involves issues of national independence, prestige and pride.

One method adopted by the Foundation originated from the request of the representative of a South Asian country for help in strengthening the library of his Ministry of Foreign Affairs, which was quite inadequate for present-day needs. Some twenty-five countries have now sought such assistance, which has been provided for twenty-one after discussion with the ambassador or a senior official of each country, while representatives of more than twenty countries took the initiative in requesting further aid and guidance in training their young diplomats. In consequence, the Carnegie Endowment for International Peace formulated a programme for creating a corps of diplomats thoroughly conversant with the practices and procedures of modern international relations. Under this programme, to which, since April 1959, the Rockefeller Foundation has contributed 525,000 dollars, sixteen Fellows nominated by the governments of fifteen newly independent countries have been sent to the School of International Affairs at Columbia University or the Geneva Graduate Institute of International Studies, a part-time seminar for younger diplomatic officers in embassies in Washington has been conducted by members of the Johns Hopkins University School of Advanced International Studies, and a training programme arranged in New York for young representatives of missions to the United Nations.

A training and study programme at a more advanced level, involving a year of intensive study, has also been arranged at Harvard University and, taken together, the two programmes, to which the Foundation has contributed 668,200 dollars, represent a carefully designed attempt to meet the urgent needs of countries which almost overnight must develop a structure of government that requires competent and qualified foreign service personnel. No one would suggest that such measures alone will solve the whole problem. Stability and security must await the slow process of educating whole nations in the difficult art of responsible government, but the Rockefeller Foundation is clearly making a valuable contribution to a vital element in the provision of leaders of understanding and imagination.

There are other fields also in which the Rockefeller Foundation is seeking to meet these needs of the emerging and under-developed nations. Mr. Harrar refers to its efforts in dealing with the language problems in Peru and Bolivia, and intellectual communication between Latin America and the United States and among the Latin American countries themselves; but one of the most interesting sections of his review is that in which he describes the growing interest of the Foundation in the agricultural development of Africa, and particularly its efforts to enable the existing agricultural institutions of Africa to provide well-rounded organic programmes of agricultural research. During 1960 the Foundation appropriated funds for this purpose to four institutions of university-level and one college of technology, as well as making awards to several experimental stations in East Africa for research on cereal crops

and for studies of livestock physiology. Thus, 41,300 dollars went to the University of Khartoum faculties of agriculture and veterinary science, and 75,000 dollars to the University College of Rhodesia and Nyasaland for intensive studies of micro-organisms and their capacity for fixing nitrogen and on the range and adaptability of native species of grasses and their capacity to provide optimum grazing conditions for cattle. Lovanium University, in the Congo, received 26,300 dollars for the purchase of X-ray equipment for the study of the mineral content of tropical soils and the influence of weathering on them. The Department of Agriculture at the University College of Ibadan, Nigeria, received 40,000 dollars to accelerate studies of production economics and of the marketing, preservation and storage of produce and for studies of grass management systems for Ndama cattle, while 42,750 dollars went to Kumasi College of Technology for teaching and research equipment for agricultural science. Grants to the East African Agricultural and Forestry Research Organization, the Kenya Department of Agriculture and the East African Veterinary Research Organization brought the Foundation's total contribution in this field in 1960 to 405,350 dollars.

This is only a fraction of the Foundation's appropriation of 6,713,314 dollars for programmes in agricultural sciences in 1960 out of a total appropriation of nearly 33 million dollars, and of this some 2·6 million dollars were for the operating programmes in this field. It compares with appropriations of 10·25 million dollars in medical and natural sciences, nearly 4·5 million dollars for the humanities and nearly 4 million dollars for the social sciences; but it is interesting to note something of the same pattern in the allocation of grants by the Nuffield Foundation described in its latest annual report. Grants allocated for the Commonwealth Overseas amounted to some £375,000, including £250,000 for the proposed medical school at the University College of Rhodesia and Nyasaland; and its fellowship and scholarship schemes of £226,000 from grants which totalled £2·26 millions during the year, of which nearly £90,000 was for scientific research and just over £300,000 for medical research, the total amount for the Commonwealth Overseas being £600,000. While the Nuffield Foundation is clearly not directing the same effort to agricultural science as the Rockefeller Foundation, both Foundations are manifestly placing their emphasis on the support and fostering of excellence.

There are, indeed, passages in Mr. Harrar's review to the Rockefeller Foundation which seem to overlook the importance of an educated democracy and the dangers which can attend the use of the media of mass communication by the demagogue in the more backward countries. By and large, however, the efforts of the Rockefeller Foundation to help the emergent nations to develop the type of leadership and trained men and women they need are soundly and imaginatively conceived, and well designed to achieve their purpose if only they are given reasonable time.

SURVEY OF ANTIBIOTIC LITERATURE

Antibiotica

Herkunft—Arten—Eigenschaften. Von Prof. T. Korzybski und Prof. W. Kurylowicz. Pp. xix+1105. (Jena: Gustav Fischer Verlag, 1961.) 130 D.M.

THIS book, first published in Warsaw in 1955, now appears in German after being brought up to date. Its object is to summarize in a systematic way the enormous amount of antibiotic literature which has appeared since the discovery of penicillin. The authors believe that it will help in the very difficult problem of quickly identifying an antibiotic met with in antibiotic research if it has already been described in the literature.

In the introduction, the authors point out that it is not possible yet to classify antibiotics from a chemical point of view as so many of the antibiotics described are of unknown chemical structure. Hence, they chose to classify antibiotics according to their sources. There are seven chapters dealing, in turn, with antibiotics from bacteria, Actinomycetes, Fungi Imperfecti, Basidiomycetes and Ascomycetes, lichens and algae, higher plants, and animal sources. The first three chapters are further subdivided, Chapters 1 and 3 on the basis of a sub-classification of the source, while in Chapter 2, which comprises more than half the book, the principal basis of subdivision is the type of antibiotic activity displayed.

Despite the fact that it is not possible to classify all antibiotics from a chemical point of view, there are a few clearly recognizable chemical groups and these are treated together by the authors. Thus Section C of Chapter 1, which deals with antibiotics produced by bacilli, contains a useful general discussion of polypeptide antibiotics, and the polypeptide antibiotics produced by Actinomycetes are dealt with in a section of Chapter 2. This chapter also includes a section dealing with the Macrolide group of antibiotics (Erythromycin, Magnamycin, etc.) and the antibiotics active against moulds are divided into the polyene antibiotics and others, and a brief general discussion of the polyene antibiotics is given.

From the narrow point of view of the identification of a particular antibiotic, a basic classification on, for example, extractability into various types of solvent might have been preferable to that adopted. But the authors have a rather broader purpose; they appear to have made satisfactory use of order wherever it exists, and they have probably produced as good and workable a classification as is possible at the present time.

The articles on individual antibiotics aim at giving the name and description of the source; a summary of culture media, methods of cultivation, isolation and purification; the physical and chemical properties; the antibiotic spectrum; the toxicity found in animal experiments; and the ultimate importance in medical research such as clinical application, if any. Naturally, many descriptions are not complete, either due to the absence of information in the literature or because statements in the literature are not sufficiently precise.

The preface is dated June 1960, and the latest reference appears to be dated September 1959. Allowing for the great amount of work that must have gone into the preparation of the book for the press, this appears to represent as small a time-lag as could be hoped for, so that the book may be con-

sidered satisfactorily up to date. Extensive tests have failed to reveal any omissions so that the book's coverage of its field appears to be complete.

Besides the pages of contents, which list all antibiotics described by name, there are an author index, an index of antibiotic names and an index of sources. With these aids, no reader should experience any difficulty in finding what he wants.

I have read a number of individual articles dealing with antibotics and in every case the article was found to present a very satisfactory and accurate summary of the information available in the literature.

The bibliographies are very good and wherever possible references are included to review articles and monographs which contain comprehensive bibliographies.

Careful perusal of the book from all points of view, therefore, suggests that it is destined to become an important work in the field of antibiotic research. The price will, unfortunately, probably prevent many individual research workers from buying it, but it is a book which should be included in any library which makes a serious attempt to cover antibiotic interests. In such libraries it will almost certainly prove to be in constant demand. B. K. Kelly

TRAITÉ DE ZOOLOGIE

Traité de Zoologie

Anatomie, Systématique, Biologie. Publié sous la Direction de Pierre-P. Grassé. Tome 5, Fascicule 1 : Annélides, Myzostomides, Sipunculiens, Echiuriens, Priapuliens, Endoproctes, Phoronidiens. Pp. viii + 1116 + 5 planches. Fascicule 2 : Bryozoaires, Brachiopodes, Chétognathes, Pogonophores, Mollusques (Généralités, Aplacophores, Polyplacophores, Monoplacphores, Bivalves.) Pp. 1168 + 5 planches. (Paris : Masson et Cie, 1960.) Broché 180 NF.; cartonné 190 NF. each.

THE present welcome volume, in two fascicules, deals with a number of separate and not all closely related groups of the lower invertebrates as indicated in the titles. Both fascicules contain almost precisely the same number of pages. Each has a short list of errata, a detailed and excellent index and a full, expanded table of contents, which appears at the end. They are both very well illustrated, each with 5 coloured plates and Fasc. 1 with 904 and Fasc. 2 with 954 text figures. While many of the illustrations are borrowed but redrawn, a remarkably high proportion are original and they reach a very high standard, adding much to the usefulness and attractiveness of the volume.

Apart from the editor, Prof. Pierre-P. Grassé, ten authors contribute to Fasc. 1, two of whom, one from Brussels and one from Algiers, come from outside France, and all are experts in their own fields. The majority of the text figures are line blocks and 18 have one or in some cases two colours introduced, and this is a commendable practice since it aids easier and quicker interpretation ; Fig. 857 in the present copy has the colour out of register, a point that is noted because the others are so exact. Twenty-one figures are in half-tone. The text of the chapter on the Phoronidea finished on p. 1053 and is followed by a number of addenda which amplify or bring up to date points in the preceding sections and then by the index and table of contents. Curiously, these pages are numbered MLV-MCXVI and the figures I–XXIX, but in Fasc. 2 the pagination and numbering of text figures carry on with p. 1054 and Fig. 876 as if the pages numbered in Roman numerals did not exist. In some places, for example, p. 34 kefersteinii, p. 120 dumerilii, etc., the double 'i' is employed instead of the now generally accepted single 'i' and on p. 994 the former name is misspelled Kleferstein. Two forms, *Histriobdella* and *Branchiobdella*, the names of which suggest leeches, with which, *inter alia*, they have been placed and the systematic position of which has been subject to discussion, are here regarded as highly modified and as a parasitic polychæte and a parasitic oligochæte respectively. Another anomalous form, *Acanthobdella*, some of the characters of which suggest Oligochæte affinities, has now been placed in the Acanthobdelliformes, a new order of the Hirudinea. An anecdote well known in Great Britain will now be lost sight of as it has been shown, p. 846, that *Golfingia macIntoshii* is a synonym of *G. vulgare*.

Ten authors are also concerned with Fasc. 2, and of these three come from outside France, two from Copenhagen and one from Leningrad. In this fascicule there are only 4 text figures with colour introduced, but there are 63 half-tone blocks and of these 8 occupy the whole page and are of wash drawings, each showing about a dozen species. The rest of the figures are in line block. The first class to be treated in the longest chapter is the Bryozoa, falling into the two sub-classes proposed by G. J. Allman in 1856, the Phylactolaemata, all freshwater, and the Gymnolaemata, marine. The latter were divided into three orders Cyclostomata, Cheilostomata and Ctenostomata by G. Busk in 1852 and this grouping was accepted for more than seventy years. In 1926 F. Borg showed that the Cyclostomata (his Stenolaemata) were more widely separated from the other two than these were from one another, and E. Marcus in 1938 placed them as sub-orders of the Eurystomata. This classification, with its corollaries to include fossil forms, has been adopted in the present work. It is rather strange to find Allman's monograph on fresh-water Polyzoa appearing under the bibliography of the Gymnolaemata.

About half-way through the volume is a most interesting chapter on the Pogonophora. In 1914 M. Caullery described an enigmatic tubicolous worm-like creature with numerous tentacles, hence Pogonophora (πωγων, a beard), collected by the *Siboga* Expedition. The material was not very well preserved and so a completely satisfactory account could not be given and indeed it was suggested that this animal, called by its discoverer *Siboglinum weberi*, might only be the anterior part of an animal since no trace of an alimentary canal was found. It was considered that the creature might be allied to the Enteropneusta and so M. Caullery contributed a chapter on it in volume XI of the *Traité*, where indeed it belongs, in 1948. Since then, and particularly by Prof. A. V. Ivanoff of Leningrad, a great deal of additional information has been furnished and now 11 genera with 43 species have been described from better-preserved material. The editor has fortunately got Prof. Ivanoff to contribute the chapter on the Pogonophora. It is the first account and survey available to the general zoologist. It provides a very complete account of the anatomy, histology and embryonic development, and in addition to the ordinary text figures, mostly original, there are three coloured plates. These depict the animals in three dimensions. It is clear that they are deuterostomes with a single cœlomic sac in the protosome, which sends canals

into the tentacles, a pair of cœlomic sacs in the mesosome and another pair in the metasome. It is concluded that they are related to the Stomatochorda and particularly to the enteropneusts. This connexion with the early Chordata together with the absence of an alimentary canal render them an important and intriguing class.

The next class calling for comment is the Monoplacophora, a name given by N. Odhner in 1940 to a group of Palæozoic Mollusca until then grouped with the Patellacea. In 1957, however, H. Lemche described a living form, *Neopilina galatheae*, referable to the group which had been collected five years previously by the *Galathea* Expedition from west of Costa Rica at a depth of 3,570 metres. Afterwards, H. Lemche and a colleague, K. G. Wingstrand of Copenhagen, gave a full account of it in the *Galathea Reports*. The editor of the volume, Pierre-P. Grassé, got these two authors to provide an account, which he translated, illustrated by a number of new figures, so that again we have the first general account of a most remarkable animal from the original describers. The well-developed cœlomic cavities are described as consisting of three pairs—the pericardial pair, the body pair which lie dorsally and are extensive, and the gonads. The apparent metamerism of a number of organs (muscles, nerves, gills, blood vessels, nephridia and gonads) is well marked and considered by the authors to be comparable with that in Annelida and Arthropoda. These points raise interesting problems concerning the relationships and derivation of the primitive molluscs. While the chapter was in the press another mollusc, for the present referred to the same genus *N. ewingi*, was reported (Clarke, A. H. and Menzies, R. J., *Science*, 1959), and although little information regarding it is available it seems to offer certain divergences from *N. galatheae* which will have to be taken into account before coming to definite conclusions on the problems above mentioned.

Here, then, is another addition to this widely used text-book that is indispensable to every zoological laboratory. It reaches the high standard in both text and illustration set by its predecessors and is indeed a noteworthy and commendable volume.

C. H. O'Donoghue

POLYMER CHEMISTRY

An Introduction to the Organic Chemistry of High Polymers

By Prof. Carl S. Marvel. Pp. viii+82. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 36*s*. net.

THIS book consists of notes originally assembled for use in connexion with the Humble Lectures in Science, delivered by Prof. Marvel in 1956. It is intended for those just starting research in polymer chemistry.

Most of the introductory texts at present available in polymer chemistry attempt to be reasonably comprehensive in their choice of topics: this one is strictly limited to the organic chemistry of high polymers. Neither the physical chemistry of polymers and their solutions nor physicochemical methods of studying polymers are considered. After a very brief description of methods for the characterization of polymers, the author proceeds to a systematic consideration of various types of condensation polymerization. Both free radical and ionic methods of addition polymerization are then discussed, followed by chapters on co-polymerization and di-ene polymerization. A short account of reactions of polymers concludes the book.

As is only to be expected in a book of this size, individual topics are not dealt with in much detail. There are a number of occasions, however, where the discussion is so brief as to lack clarity. Particular examples are the section on the mechanism of free radical addition polymerization and the introduction to the section on co-polymerization.

The most serious limitation of the book is that it is already five years out of date. It is a pity that so little attempt seems to have been made to include work done after 1956. Because of the rate at which polymer chemistry is advancing, this leads to some important omissions. For example, topics which are omitted or receive only the briefest mention include stereospecific polymerization, polyaldehydes, polycarbonates, polymer stability and degradation, radiation methods, and new methods of anionic polymerization. Quite apart from their importance, these developments in the forefront of the subject are those most likely to arouse the enthusiasm of a beginner, which is surely one purpose any book of this kind should serve.

There are few trivial misprints. More serious is the appearance of pentavalent carbon on pages 47 and 48, unhappily in a place where the unwary reader might be misled into thinking that Friedel–Crafts-type catalysts used in cationic polymerization are covalently bound to the polymer chain. On page 47 is the statement that in every case of co-catalysis in the initiation of cationic polymerization the real initiator is believed to be a proton. This neglects the important cases where there is good evidence that carbonium ions derived from the solvent are chain initiators.

The book is, in general, attractively printed and strongly bound. At a cost of fivepence a page, however, it must be one of the most expensive chemistry books available.

I. C. McNeill

QUANTITATIVE BIOLOGY

Blood and other Body Fluids

Analysis and compilation by Philip L. Altman. Edited by Dorothy S. Dittmer. (Biological Handbooks.) Pp. xvii+540. (Washington, D.C.: Federation of American Societies for Experimental Biology, 1961.) 10 dollars.

NUMERICAL information is becoming of ever-increasing importance in all branches of biology. While physicists, chemists and engineers started long ago to compile handbooks of data, the collection of biological figures is of relatively recent date. The first major systematic effort in this direction were the *Tabulae Biologicae* which were initiated in the 1930's. Some ten years ago the United States National Academy of Sciences and the United States National Research Council began to sponsor a series of biological handbooks which has so far led to the publication of ten volumes. Some deal with blood, nutrition, metabolism, general biology, respiration and circulation, and five others with various aspects of toxicology and pharmacology (acute toxicity, antibiotics, insecticides, tranquillizers and fungicides). The present volume is based on these earlier handbooks and is the first to appear under the general direction of the Committee of Biological Handbooks of the Federation

of American Societies for Experimental Biology. The material is presented in the form of tables, graphs, diagrams, nomograms and line charts. Three hundred and eighty investigators have taken part in the compilation and much trouble has been taken to obtain accuracy, to eliminate misprints and to exclude material of questionable validity. Apart from blood, fields covered include urine, lymph, hæmolymph, cerebrospinal fluid, body water, various secretions of the digestive tract, reproductive secretions, milk, sweat, sebum and the fluids of the ear and eye. Among the species, man occupies the first place, but the information extends to numerous other vertebrate and invertebrate organisms.

While the data of physical and chemical reference books are usually constants which have been measured with a known accuracy, biological data are, as a rule, not so definable: they are not constants and their scatter is in many cases not sufficiently known. The figures given in the book are therefore bound to be of varying degrees of reliability, but this difficulty has been effectively met by detailed references to the sources of information. In fact the references given may well prove to be one of the most valuable features of a very useful mine of factual information.

H. A. Krebs

A NEW STANDARD WORK ON COCOA

Cocoa

By D. H. Urquhart. Second edition. (Tropical Agriculture Series.) Pp. xxv+293+129 plates. (London: Longmans, Green and Co., Ltd., 1961.) 40*s*. net.

THIS book was originally published in 1955 and two additional impressions were issued in 1955 and 1956. The new (second) edition attempts to bring the subject-matter up to date. It contains one entirely new chapter (on manuring) and several of the chapters of the first edition have been combined in the second. The total number of pages has thus been increased from 230 to 293. The book is one of a series on tropical agriculture of which five volumes have already been published. The author of *Cocoa* was formerly director of agriculture of Ghana, West Africa, and adviser in cocoa-growing to Messrs. Cadbury Bros., Ltd., Bournville, Birmingham, England.

The book includes all the detailed information needed by practical cocoa farmers, as well as students and agricultural extension workers. Its usefulness is greatly enhanced by the excellent illustrations and the tables of data. Much progress has been made in the cocoa industry since the first edition of this book appeared in 1955, particularly in the control of pests and diseases of the cocoa tree, and the detailed specification of the atmospheric and soil environment necessary for maximum production of cocoa. Thus, in the chapters concerning soils and manuring, the results of researches carried out in recent years in Ghana by the late C. F. Charter and in Belgian Congo by M. V. Homes have been incorporated into the new edition. The establishment, maintenance and costings of the cocoa plantation, the rehabilitation of the derelict and unremunerative plantation, and the preparation and grading of the crop for market, receive detailed consideration in no less than seven chapters and nine appendixes, spanning a total of 159 pages or 50 per cent of the total number of pages comprising the book, thus stressing the essentially practical outlook. That Urquhart's book is providing a much-needed standard work on cocoa and one which is a worthy successor to the classical works on the subject written by C. J. J. Van Hall and by J. H. Hart, which have long been out of print, is indicated by the fact that the first edition and its two impressions were exhausted after only five years had elapsed. The need for a Spanish version has already become manifest, and the task of translation has been undertaken by the Inter-American Institute of Agricultural Sciences at Turrialba, Costa Rica. Publication of the Spanish edition is expected shortly; it should add to the increasing demand for the book by making it available to cocoa growers in tropical America.

LONDON LIBRARIES

The Libraries of London

Edited by Raymond Irwin and Ronald Staveley. Second, revised edition. Pp. 332. (London: The Library Association, 1961.) 36*s*.

THE first edition of this work, published in 1949, was based on lectures delivered during a course at the School of Librarianship and Archives, University College, London, and was intended to supplement but not replace R. A. Rye's *Students Guide to the Libraries of London*. That work has long been out of print and even the first edition of *The Libraries of London* is now largely out of date. Accordingly, the new edition attempts to present a much fuller picture of the library resources of London. All the chapters have been carefully revised and most have been largely re-written; there is a new chapter on the University College Library, and separate chapters on the University of London Library, on the British Library of Political and Economic Science, while the other university libraries are described in a separate chapter. Additional chapters deal with the music libraries, the ecclesiastical and theological libraries, the industrial libraries, technical and professional libraries and those of the learned societies, and of the London County Council, while that on the Public Record Office is omitted. Apart from the London Library, no attempt is made to describe the commercial and subscription libraries, and likewise there is no reference to the libraries associated with the offices of the great national newspapers.

The individual chapters are the work of different authors, all authorities in their field, but what is gained in authority is sometimes offset in part by lack of balance, and diversity of scale and treatment. That on the Science Museum Library, by the late H. T. Pledge, is frankly episodical, and besides its historical or descriptive lacunæ, it has scant reference to future developments. The Patent Office Library is more adequately covered, but there are some surprising omissions in the chapter on Government Departmental libraries, while that on industrial libraries is scarcely of greater value than as a reminder of the existence of such libraries; it is content to list and describe those of the British Aluminium Company, the Cement and Concrete Association, and the British Paper and Board Industry Research Association. Nevertheless, used with imagination, this book, produced in accordance with the high standards which are customary with the Library Association, forms a most useful and convenient guide to the bibliographical resources of London.

R. Brightman

Sensation and Perception

A History of the Philosophy of Perception. By D. W. Hamlyn. (International Library of Philosophy and Scientific Method.) Pp. xi+210. (London: Routledge and Kegan Paul, Ltd.: New York: The Humanities Press, 1961.) 25*s*. net.

THERE is something very refreshing about this book. It deals with a complex theme without reducing the reader to fatigue and his patience to zero. Broadly, the author takes as his 'matter' the trio—sensation, perception, judgment—and subjects them to a careful historical analysis. In a word, we are told how these concepts have evolved with time, not forgetting that ideas need verbal expression for them to come into being. However, the absence of such terms is not proof positive that the 'germ' was not alive in the mind of philosophers, at any given epoch, or even perhaps at some previous period.

We begin with the classical figures, followed by a lively discussion of Hellenistic thought. (Incidentally, the heading to Chapter 2 on page 31 looks very odd with this adjective spelt with one 'l'). Next come chapters on the seventeenth century, rationalists, empiricists, Kant, Hegel, idealism, nineteenth-century sensationalism, the reaction, the twentieth century, conclusion. There are an excellent bibliography and an adequate index.

The connexion with *Gestalt* theory is well handled. Perception, given that the whole is more than the sum of its parts, needs no learning "because it is innately determined". Perception, if passive, tends to sensation; if active, to judgment. The former is unsatisfactory in various ways, especially to the scientist, who uses the second almost subconsciously. What must be avoided, as Wittgenstein remarked, is the introduction into this study of one's own experiences. In the pre-perceptual sense at least, there is no private language.

The style is pleasant and lucid: fewer italics would have made it still more agreeable without any loss of emphasis. F. I. G. RAWLINS

Analytical Chemistry of the Rare Earths

By Dr. R. C. Vickery. (International Series of Monographs on Analytical Chemistry, Vol. 3.) Pp. viii+137. (London and New York: Pergamon Press, 1961.) 40*s*. net.

THE rare earths, as defined by the author of this invaluable little volume, comprise the lanthanon series to lutetium with the addition of yttrium and scandium.

The analytical examination of mixtures of these elements has been rendered far less time-consuming in recent years by the application of the newer technique of X-ray fluorescence analysis. Radioassay following neutron irradiation now permits the determination of millimicrogram quantities, and controlled ion-exchange experiments have enabled the chemist to separate the individual rare earths in a very pure form. All these modern methods are described lucidly in this monograph, which also contains the most detailed description of the more classical, but still eulogized, absorption spectrum technique for the detection and estimation of these intractable elements. Conventional emission spectrography is not neglected by the author, for despite the complexity of the spectra given by these elements, there are occasions where the arc, or preferably the spark technique, can give the most useful quantitative information. A table is given of the main persistent lines of the rare-earths emission spectra, and various internal standard line-pairs are also presented in tabular form for specified concentration ranges.

Polarographic methods as applied to the unique cases of samarium, europium and ytterbium are described briefly in the appendix.

The earlier chapters deal with the more classical methods of gravimetric and volumetric analyses, and here the student will find a wealth of information on chemical separational procedures. The analysis of ores, the separation of calcium and iron, the usages of selective organic reagents such as Beck's pyrocatechol, complications attending the Group III hydroxide precipitations, are all described with much attention to laboratory detail. Although quantitative methods for the determination of the rare earths are now almost wholly instrumental, the uses of oxalate, iodate, bismuthate and complexan reagents are well worthy of recapitulation. In retrospect, we join with the author in expressing unqualified admiration of the skill, patience and ingenuity shown by the early chemical research workers who isolated, albeit in crude form, the first rare-earth elements.

This monograph has much to commend it to the academic student, but for the rare-earth chemist it is most certainly an essential acquisition and will prove a valuable laboratory companion. D. T. LEWIS

Paper Electrophoresis

A Review of Methods and Results. By L. P. Ribeiro, E. Mitidieri, and O. R. Affonso. Pp. xii+463. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1961.) 72*s*.

THIS book by Ribeiro, Mitidieri and Affonso represents a welcome and thorough classification of the already voluminous literature on ionography. There is a wealth of references, 3,200, some of which have been published only in the more esoteric medical journals.

The first part of the book includes chapters on the theory, methods and apparatus. The theoretical chapter is written with great clarity and will be very useful to beginners in this field. Even Ohm's law is included and there is a useful example on the results of Joule's effect. "When high voltages are used there will naturally be an increase in current causing an elevation in temperature which may be so great as to ignite the paper."

In the subsequent part of the book on applications the emphasis is on proteins, but there are also chapters on hormones, vitamins, carbohydrates, nucleic acids, amino-acids and peptides, a sketchy chapter on inorganic substances, one on immunochemistry and one on other applications which deals with topics as diverse as amines, dyes and venoms.

The large bibliography means that the treatment afforded individual references varies considerably throughout the book, ranging from minute practical detail to just the reference itself. The book has few printing and translation errors and the diagrams are well produced. Two points might, however, be mentioned: on page 211 the structural formula given for the sugar unit after oxidation with periodic acid appears to be that for an α-diketone rather than for a di-aldehyde as suggested in the text, and for greater clarity the cyclohexane rings which appear in some of the formulæ throughout the book might, where appropriate, be amended to aromatic rings.

The book will undoubtedly be a valuable asset to biochemists, chemists and clinicians, particularly as there are more than three hundred references per ounce. G. V. F. SEAMAN

XUM

A MICROBIOLOGICAL EXAMINATION OF SOME CARBONACEOUS CHONDRITES

By Dr. GEORGE CLAUS
New York University, Medical Center, New York
AND
Prof. BARTHOLOMEW NAGY
Department of Chemistry, Fordham University, New York

MICROSCOPIC-sized particles, resembling fossil algae, were found to be present in relatively large quantities in the Orgueil and Ivuna carbonaceous meteorites. No such particles were found in two ordinary stony meteorites, namely, in Holbrook and in Bruderheim.

There are nineteen known carbonaceous chondrites, all of which were observed to fall. Orgueil fell in southern France in 1864, Ivuna in Central Africa in 1938, Mighei in Russia in 1889, and Murray in the United States in 1950. Carbonaceous chondrites contain water and a few per cent of organic matter.

Wiik[1] has shown that carbonaceous chondrites may contain as much as 20 per cent water. On the basis of his hydrogen isotope work, Boato[2] concluded that a large portion of this water in Orgueil and Ivuna is of extraterrestrial origin. Carbonaceous chondrites have thin fusion crusts. Mueller[3] and others found that after the development of the indigenous, hydrous-mineral components, the interior of these meteorites was not subjected to high temperatures. The interiors of some of the carbonaceous meteorites apparently have not been heated to temperatures higher than approximately 200° C.

Berzelius[4] and Wöhler[5] extracted organic matter from carbonaceous chondrites. Berthelot[6] obtained saturated hydrocarbons from a sample of Orgueil. In more recent years, Mueller extracted the Cold Bokkeveld meteorite and obtained organic matter which had high oxygen, nitrogen and sulphur content. Calvin[7] suggested that a compound resembling cytosine might be present in Orgueil, and Briggs[8] presented analyses which may indicate the presence of purines. Nagy, Meinschein and Hennessy[9] analysed hydrocarbons from Orgueil with a mass spectrometer and observed certain spectral features which resembled those of biogenic hydrocarbon mixtures.

Urey and Craig[10] concluded that the carbonaceous meteorites are typical members of the high iron group chondrites. These investigators reported that serpentine-type minerals and magnetite are present in carbonaceous chondrites. Kvasha[11] suggested that chlorite, a hydrous layer-lattice silicate, is a mineral component in Staroye Boriskino.

Five carbonaceous chondrite samples were examined during this work. One Orgueil sample (*A*), Ivuna and Mighei, were obtained from the American Museum of Natural History in New York, and another sample of Orgueil (*B*) was received from the U.S. National Museum in Washington, D.C. The sample of the Murray meteorite was obtained from the Institute of Meteoritics, University of New Mexico, Albuquerque, New Mexico. One of the two non-carbonaceous chondrites, Bruderheim, was received from the Department of Geology, University of Alberta, Edmonton, Alberta, and the other, Holbrook, was received from the American Museum of Natural History.

Grains of the meteorite samples were crumbled in water or in glycerol on glass slides. A microscopic examination of the two Orgueil samples and of Ivuna revealed the presence of particles which were dissimilar in morphology to any known mineral form. The 'organized elements' resembled, but were not identical to, the morphology of certain species of algae which live in water. (The aquatic algae are capable of producing hydrocarbons[12] comparable with those previously found in Orgueil[9].) One form of the organized elements was morphologically dissimilar to any known terrestrial organism. The organized elements did not show birefringence in polarized light, but they did show fluorescence (yellow) in ultraviolet light (Fig. 1). A few specimens of microbiological contaminants (common, airborne bacteria, algae, etc.) were present in the carbonaceous as well as in the non-carbonaceous meteorites. Organized elements were abundant in the Orgueil and Ivuna carbonaceous chondrites but were absent in Holbrook and in Bruderheim.

Somewhat similar but poorly defined particles were found in Murray and Mighei. Their lack of structure precluded identification with the organized elements from Orgueil and Ivuna (see Table 1).

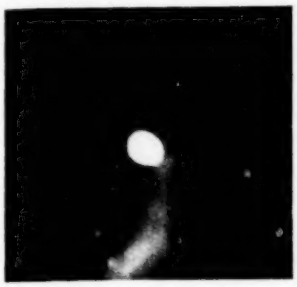
Five types of organized elements have thus far been observed:

(1) Small, circular forms (Fig. 1), surrounded by double walls which showed thickening and occasional sculpturing with protuberances. Their diameter varied between 4 and 10μ and they appeared yellowish-green in transmitted light. The interior of the organized elements appeared to be either homogeneous or it was granulated.

(2) The second type (Figs. 2 and 3) resembled the first one; however, the surfaces of these organized elements were covered with spines, with other appendages or with a furrow. The appendages usually appeared to be tubular in shape, and occasionally they penetrated through the walls. The diameter of the appendages varied between 0·5 and 2μ, and that

Table 1. Organized Elements and Contaminations in Five Meteorites
(The numerical values represent the number of particles in 1 mgm. of sample)

Type of particles	Orgueil (*A*)	Orgueil (*B*)	Ivuna	Holbrook	Bruderheim
Organized elements	1650	1700	1680	—	—
Apparent contaminants					
(*a*) Bacteria	27	34	31	22	48
(*b*) Algae					
Achnanthes microcephala	1	—	—	—	2
Navicula sp.	—	1	—	—	—
Nitzschia palea	—	—	1	—	1
Trachelomonas volvocina	—	—	—	—	1
(*c*) Others					
Conidiospore	—	—	—	—	1
Sponge spicule	2	—	—	—	—
Starch granule	1	—	—	—	—
Cellulose fibre	4	6	6	22	50
Total contaminants	35	41	38	44	103

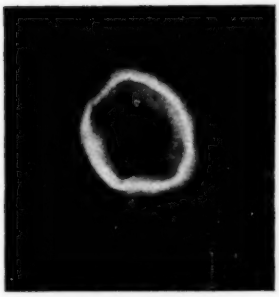


20μ

Fig. 1. Fluorescence effect in an organized element, type 1, in ultra-violet light

10μ

Fig. 2. Organized element of type 2 in the Orgueil meteorite



10μ

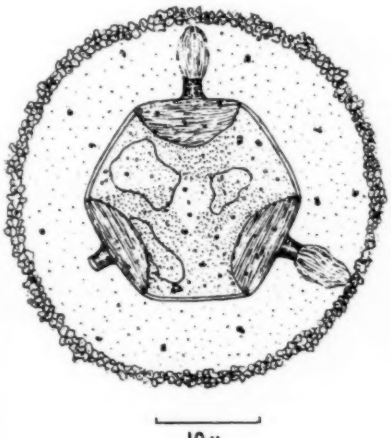
Fig. 3. Organized element of type 2 as seen under the phase-contrast microscope. Note internal structure of the organized element

of the organized elements was between 8 and 30μ. Solid appendages had club-like structures on their distal ends. Spines were arranged in pairs around the appendages. The furrow started at the single appendage on the apex of the organized elements.

(3) The third type of the organized elements had shapes like shields. They had no appendages. Their walls showed marked thickening and sculpturing; their average diameter was approximately 15μ.

(4) Cylindrical forms were seen which had thick walls and finely sculptured wall surfaces. Their length was approximately 20μ. their width was 10–12μ.

(5) An apparently hexagonal form was also observed (Fig. 4). This organized element may contain, however, 10–12 surfaces. Three of the surfaces were considerably thicker than the others and they served as the bases of the three, thick, tubular protrusions. The organized element seemed to be surrounded by a structureless halo, which was interrupted only by some matter that was protruding from two of the appendages. There were three vacuole-like and irregularly shaped bodies in the interior of the organized element.



10μ

Fig. 4. Sketch of organized element of type 5. (Drawing is used because the original forms were not suitable for taking photographs)

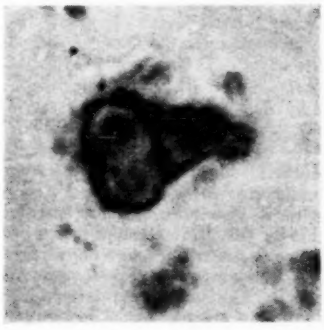
The Orgueil meteorite samples contained each of the five organized element types. Only the first three types were present in Ivuna. Types 1 and 2 were by far the most abundant in both Orgueil and Ivuna.

There were other morphological features which should be mentioned. Several of the organized elements appeared to undergo 'cell-divisions'. Constrictions occurred in the middle portions of the elongated bodies in one form of this phenomenon, proceeding toward the interior and suggesting the formation of two 'daughter' bodies (Fig. 5). Several organized elements showed distortion and rupture of the walls. Often the interior appeared to be spilling out through the fractured walls.

Attempts were made to identify the chemical components of the organized elements with biological staining techniques; chlorzink-iodine, toluidine blue, methylene blue, Janus green *B*, Feulgen, Gridley, acid-carmine, periodic acid–Schiff, hæmatoxylin eosine and ninhydrin gave colour reactions. The reliability and the significance of the colour reactions in the organized elements are not known. It is interesting to note, however, that diffuse nuclear-staining material was found in the organized elements (Fig. 6).

From these morphological, optical and staining results we have interpreted the organized elements as possible remnants of organisms.

Although the origin of these organized elements cannot be answered at this time, indications of their



10μ

Fig. 5. Organized element of type 2 resembling 'cell division'

10μ

Fig. 6. Organized element of type 1 after Feulgen staining; note indications of diffuse nuclear material

origin may be summarized in the following observations:

(1) The high concentrations of the organized elements in Orgueil and in Ivuna could have developed only in an environment where moisture was continuously present for a prolonged period of time; yet the meteorite samples were kept in dry museum storage and would have disintegrated if subjected to an aqueous medium. Furthermore, they were outdoors for only a few hours after they had fallen. There are only traces, if any, of capillary water retained in the meteorites.

(2) The organized elements, types 1–4, resemble those species of Dinoflagellates or Chrysomonads which live only in water (in the sea or in lakes, and do not occur in the soils).

(3) Types 1–4 appear to be similar to but not identical with known terrestrial species. The fifth type of the organized elements is entirely dissimilar in its morphology to known terrestrial form.

(4) Terrestrial organisms resembling types 1–4 of the organized elements develop cysts under unfavourable conditions (such as a drying or dry environment). Only two or three of the organized elements in Orgueil and in Ivuna showed structures resembling cysts.

(5) The fact that the same types of organized elements were present in both Orgueil samples which fell in the temperate climate of southern France, and in Ivuna, which fell seventy-four years after Orgueil in an arid, tropical region of Central Africa, renders unlikely contamination with morphologically identical, locally derived, micro-organisms.

The morphological similarities are of interest in the light of unpublished results of one of the authors[11] which indicate a chemical similarity between Orgueil and Ivuna.

We are of the opinion that these observations suggest that the organized elements may be microfossils indigenous to the meteorite.

We would like to express our appreciation to Prof. Douglas J. Hennessy, of Fordham University, and to Dr. Warren G. Meinschein, of the Esso Research and Engineering Co., for their constructive criticisms. Dr. Meinschein originally proposed also a palinological examination of meteorites. We would also like to thank those persons with whom these results have been discussed: Prof. Harold C. Urey, of the University of California; Dr. Brian Mason, of the American Museum of Natural History; Dr. Jack Green, of North American Aviation, Inc.; Dr. C. Mervin Palmer, U.S. Public Health Service; Profs. Currier E. McEwan, Maxwell Schubert, Norman Cooper and Alan J. Johnson, of New York University Medical Center; Prof. Henrik P. deBoom, University of Pretoria, South Africa; Dr. Bela J. Cholnoky, Council for Scientific and Industrial Research, Water Research, Pretoria; and Prof. Alexander Wolsky, Fordham University. Profs. Urey, Wolsky, Hennessy, Schubert and Johnson and Dr. Meinschein read the manuscript and offered valuable advice regarding this presentation. The meteorite samples were provided by Drs. Brian Mason, E. P. Henderson, Lincoln LaPaz and Prof. R. E. Folinsbee.

[1] Wiik, H. B., *Geochim. Cosmochim. Acta*, **9**, 279 (1956).
[2] Boato, G., *Geochim. Cosmochim. Acta*, **6**, 209 (1954).
[3] Mueller, G., *Geochim. Cosmochim. Acta*, **4**, 1 (1953).
[4] Berzelius, J. J., *Ann. Phys. Chemie*, **33**, 113 (1834).
[5] Wöhler, M., *Sitzungsber. Akad. Wiss. Wien*, **33**, 205 (1858).
[6] Berthelot, M., *C.R. Acad. Sci., Paris*, **67**, 849 (1868).
[7] Calvin, M., *Chem. Eng. News*, **39** (21), 96 (1961).
[8] Briggs, M. H., *Nature*, **191**, 1137 (1961).
[9] Nagy, B., Meinschein, W. G., and Hennessy, D. J., *Ann. N.Y. Acad. Sci.*, **93**, 25 (1961).
[10] Urey, H. C., and Craig, H., *Geochim. Cosmochim. Acta*, **4**, 36 (1953).
[11] Kvasha, L. G., *Meteoritika*, **4**, 83 (1948).
[12] Fritsch, F. E., *The Structure and Reproduction of Algae*, **1**, 1 (1935); **2**, 1 (1949). (Camb. Univ. Press).
[13] Nagy, B., Meinschein, W. G., and Hennessy, D. J. (manuscript in preparation).

BRADYKININ

By Dr. G. P. LEWIS

National Institute for Medical Research, Mill Hill, London, N.W.7

BRADYKININ is a polypeptide discovered in 1949 by Rocha e Silva, Beraldo and Rosenfeld[1], who coined the name 'bradykinin' (slow-moving) because, when acting on the guinea pig intestine, it caused a delayed and slow contraction. They showed that it is formed by the action of certain enzymes, such as those in some snake venoms and trypsin, on plasma globulins. 'Kallikrein' is the name given to another group of enzymes which occur in the body and which act on the plasma globulins to form bradykinin-like activity. Much of the earlier work on these enzymes was that of Frey, Kraut and Werle, and was reviewed by them in 1950 [2]. A vasodepressor substance was shown to be present in urine[3], in pancreas[4], in blood[5] and in saliva[6], although it was not realized at that time that this vasodepressor substance was an enzyme, acting by the formation of a vasodilator polypeptide. The active principle in all these fluids was called 'kallikrein'. However, the kallikrein obtained from these various sources is not the same enzyme. Blood kallikrein, which is different from that in glands, behaves like the proteolytic enzyme plasmin[7] and is inhibited by proteolytic inhibitors[8]. The glandular kallikrein does not behave like a proteolytic

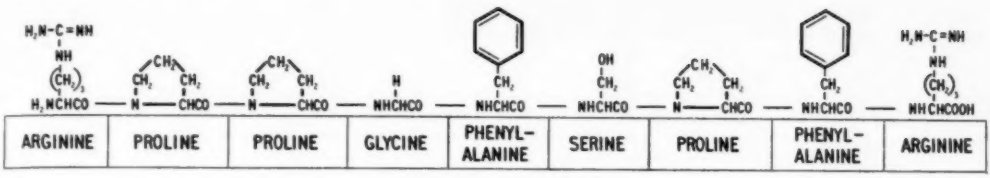


Fig. 1. Structure of bradykinin

enzyme[9]—it does not hydrolyse substrates, like casein, nor is it inhibited by proteolytic inhibitors. However, the kallikrein of the salivary gland hydrolyses certain synthetic esters, but not the corresponding amides[10]. This gives support to the theory of Rocha e Silva and Hamburg[11,12] that bradykinin is formed by an esteratic action.

Trypsin and plasmin are both proteolytic and esteratic enzymes—it may be that some of the kallikreins, particularly glandular kallikrein, are not proteolytic enzymes at all, but esterases. It is possible that the two classes of enzymes—the slow-kinin-forming enzyme and the quick-kinin-forming enzyme described by Lewis[9] are the proteolytic and esteratic enzymes respectively. Whatever the truth may be, it is certain that there are several different kinds of enzymes which act on the plasma globulins to form bradykinin, or at least peptides, which are closely related to bradykinin.

It is not certain whether the peptide formed by these different enzymes is the same in each case and therefore the name 'plasma kinin' has been suggested to cover all the peptides formed from plasma proteins in this way. For example, the name plasma kinin may be applied to the principles which are formed in blood under various conditions, such as when plasma is diluted[13] or when plasma comes into contact with a glass surface[14]. Presumably these physical actions result in activation of one of the plasma-kinin-forming enzymes which are normally present in blood as inactive precursors. Although the exact structure of all the peptides formed by these various enzymes is not known, we do know the amino-acid sequence of one plasma kinin—bradykinin. After attempts by Andrade, Diniz and Rocha e Silva[15,16], who obtained bradykinin of high activity, it was finally isolated[17], and synthesized[18,19]. From an incubated mixture of crystalline trypsin and pseudoglobulin Elliott, Horton and I isolated bradykinin using mainly the technique of elution chromatography on carboxy-methylcellulose. The peptide was eluted from the columns using a salt gradient at constant *p*H. The volatile buffer ammonium acetate was used as eluant so that the samples could be evaporated, thus avoiding contamination with salt.

The pure peptide obtained in this way contains five amino-acids in the following molar proportions: serine 1, glycine 1, phenylalanine 2, arginine 2 and proline 3, giving nine amino-acid residues or a nonapeptide.

These are present in bradykinin in the sequence shown in Fig. 1 (ref. 20). The natural and synthetic products were found to be identical[21–23].

The main pharmacological actions of bradykinin are smooth muscle stimulation, vasodilatation, increase in capillary permeability, accumulation and migration of leucocytes and production of pain.

Most smooth muscle preparations are stimulated by bradykinin even in minute amounts. The rat uterus is contracted by 0·1 ngm./ml. (10^{-10}) and the guinea pig ileum by 1·0 ngm./ml. (10^{-9}), while the rat duodenum is relaxed by bradykinin 0·1 ngm./ml. (10^{-10}). Some smooth muscle preparations like the rabbit jejunum and rat colon, which show spontaneous activity, are first relaxed and then contracted by bradykinin. Bradykinin causes bronchoconstriction in the guinea pig[24], although it has no effect on the bronchioles in the dog[25]. In non-asthmatic patients large doses of bradykinin, given as an aerosol, occasionally cause constriction of the bronchioles; asthmatics are more sensitive[26], but it seems doubtful that bradykinin is concerned specifically in asthma.

Bradykinin is one of the most active vasodilator substances known. In the cat hind limb bradykinin is as active as acetylcholine[27]. On the flow of blood in the human forearm bradykinin was found to be a more active vasodilator than histamine, acetylcholine, isoprenaline and 5-hydroxytryptamine[28]. In some of these experiments as little as 0·1 μgm. bradykinin injected into the brachial artery caused a strong vasodilatation in the whole forearm.

It has been known for some years that crude mixtures containing bradykinin cause an increase in capillary permeability[29] and pain[14]. With the availability of the pure peptide it was shown that pure bradykinin possesses both of these actions on local application[27].

In addition, bradykinin causes accumulation and migration of leucocytes[30]. This effect, however, is produced only by relatively large doses of bradykinin (1–100 μgm./ml.) and in such doses other peptides affect leucocytes in the same way.

Bradykinin thus causes vasodilatation, increased capillary permeability, production of pain and accumulation and migration of leucocytes. These are the cardinal signs of the early stages of the inflammatory response. It therefore seems likely that bradykinin plays the part of a mediator in this reaction. Menkin[31,32] originated the view that specific polypeptides may be responsible for the inflammatory response. It is possible that bradykinin is one of a group of peptides which account for this reaction as postulated by Menkin. There is no direct evidence yet that bradykinin is formed during the inflammatory reaction; however, it is possible, as suggested by Hilton and Lewis[33], that bradykinin or perhaps the enzyme which forms bradykinin may correspond to the *H*-colloid of T. H. Lewis[34] and of Krogh[35], which together with *H*-substance (presumably histamine) accounts for many of the reactions to local tissue injury. No specific antagonist of bradykinin is yet available in order to test such a hypothesis by inhibiting or preventing inflammation. It has been claimed that phenylbutazone is an antagonist of bradykinin[36]. As phenylbutazone is known to be a potent anti-inflammatory agent this would provide good evidence that bradykinin plays a major part in inflammation. However, the action of phenylbutazone is not specifically to antagonize bradykinin, and many other drugs

are partially antagonized by the large doses of phenylbutazone required (Lewis, G. P., unpublished results). Another anti-inflammatory agent—aspirin—specifically antagonizes the bronchoconstriction caused by bradykinin in guinea pigs[34]. However, here again this cannot be taken as evidence of the role of bradykinin in inflammation, as aspirin does not antagonize those actions of bradykinin which would be involved in inflammatory reactions (Lewis, G. P., unpublished results).

Although there is no direct evidence of the part bradykinin may play in the reactions to local injury, there is evidence of the part it takes as a physiological mediator of functional vasodilatation, as shown by the experiments of Hilton and Lewis[32,37-39] on perfused salivary glands. In these experiment it was found that during activation of the glands an enzyme, probably salivary kallikrein, escaped from the secretory cells into the tissue space and that this enzyme formed bradykinin, or at least a plasma kinin, when it came into contact with the plasma globulins. When the gland cells are activated, the enzyme thus escapes both into the duct to appear in the secretion and into the interstitial space of the gland around the blood vessels to form the vasodilator polypeptide. As kallikrein appears in many glandular secretions, it follows that all these glands have the plasma-kinin-forming mechanism which might therefore be the mechanism in such glands for producing functional vasodilatation. In this way—by producing an increased blood flow in the gland—bradykinin supplies the gland with the fluid required during secretion. It might not only be in glands themselves that bradykinin has such a role; in tissues which contain an abundance of glands, intestine and skin, for example, it may also account for periglandular vasodilatation. For example, in the intestine it is possible that the activation of intestinal glands leads to bradykinin formation which would bring about the vasodilatation required during the assimilation of foodstuffs.

In skin, Fox and Hilton[40] have found that human sweat contains a plasma-kinin-forming enzyme and by analogy with the salivary glands have concluded that bradykinin formation accounts for the vasodilatation in the glands during secretory activity. In the case of sweat glands bradykinin plays an additional part. As the sweat glands are in such close proximity to each other in the skin it seems likely that when the glands are activated, during body heating, the bradykinin formation which occurs accounts for the vasodilatation in the whole skin. This is the conclusion Fox and Hilton reached from their experiments on increased forearm blood-flow during body heating. In thus providing extra fluid in the increased flow of blood to form further sweat to be secreted and evaporated, bradykinin plays a part in the regulation of body heat.

The theory that bradykinin is the mediator of functional vasodilatation in glands and glandular tissue has been challenged on two points.

First, Terroux, Sekelj and Burgen[41] claimed to have dissociated glandular activity and vasodilatation in the salivary glands. As a measure of glandular activity they used oxygen uptake. However, whether oxygen uptake reflects the glandular activity quantitatively is doubtful, as these authors themselves suggest that part of the metabolism in the secreting gland occurs through anaerobic glycolysis. Their experiments show that small doses of atropine reduced the increased uptake of oxygen in parallel with secretion when the gland was activated by stimulation of the chorda tympani nerve, but did not affect vasodilatation in the gland. This problem has been examined extensively by Hilton and Lewis[38], and we have shown that after atropine, even in doses larger than those generally used by Terroux, Sekelj and Burgen, nerve stimulation still produced an output of plasma-kinin-forming enzyme. As the enzyme comes from the secretory cells, it is evident that although overt secretion is prevented, some of the biochemical processes associated with glandular activity are still stimulated. Therefore, the lack of parallelism between oxygen uptake and vasodilatation in the partially atropinized gland is not a relevant argument against the view that bradykinin is responsible for functional vasodilatation.

Secondly, Schachter[42] observed that guinea pig saliva did not act on guinea pig serum to form bradykinin and concluded that functional vasodilatation was not the result of bradykinin formation. However, Schachter also observed that the guinea pig serum used in these experiments did not form bradykinin with the saliva from cat, rat and man, but that guinea pig saliva did form a plasma kinin when incubated with the serum of other species. It seems, therefore, that it is not that guinea pig saliva does not contain the plasma-kinin-forming enzyme but that under the conditions of this particular test the serum did not react with salivary kallikrein from any species. Plasma and serum are known to contain a complex mixture of enzymes, inhibitors and activators (see ref. 43) and it may well be that in order to produce bradykinin from guinea pig serum by the action of salivary kallikrein *in vitro*, special treatment of the serum proteins is necessary.

Functional vasodilatation is a feature of glands which act intermittently, like the salivary glands, and it requires a mechanism which produces a large increase in blood flow of rapid onset and short duration. It might well be that in glands which are continuously active over relatively long periods of time, such as mammary and endocrine glands, no such functional vasodilatation occurs. This would explain the absence of plasma-kinin-forming enzyme from the secretions of such glands as ovary, thyroid and prostate[44].

The part played by bradykinin in functional vasodilatation in glands and glandular tissues and its possible role in inflammation depend on two factors which limit the area in which the reaction goes on, that is, factors which localize the reactions. First, it seems likely that the formation, the action and the destruction of bradykinin go on in the interstitial fluid. In a gland, for example, this would mean that when the gland is activated the enzyme escapes from the secretory cells into the interstitial fluid; there it acts on the globulins and forms bradykinin. The peptide dilates the blood vessels and is eliminated via the lymph. Hilton and Lewis[37] provided evidence that this actually occurs in the salivary glands and that under normal conditions it is not necessary for the peptide or the enzyme which forms it to enter the blood stream. Thus, this physiological reaction would be physically limited to the interstitial space of a gland. Secondly, there is an efficient mechanism in the body for destroying excess bradykinin. In blood, and probably in interstitial fluid, there is an enzyme which rapidly destroys bradykinin and thus controls the action of bradykinin. Without this mechanism bradykinin formation in the blood stream

would result in generalized peripheral vasodilatation and vascular collapse. This is, in fact, what might happen in certain cases of shock. The reactions of certain proteins, such as antigen and antibody in the blood stream, may activate the plasma-kinin-forming system. Bradykinin or a plasma kinin would then be formed in the blood stream and if it were associated with a reduction in the bradykinin-destroying activity of blood, it would result in vascular collapse and shock.

However, there is as yet no evidence to indicate that bradykinin plays any part in generalized reactions. All the evidence that we have suggests that bradykinin acts in local reactions; first, as the physiological mediator of functional vasodilatation and, secondly, as a mediator of the inflammatory response. In such local phenomena bradykinin might well be classified as a local tissue hormone.

[1] Rocha e Silva, M., Beraldo, W. T., and Rosenfeld, G., *Amer. J. Physiol.*, **156**, 261 (1949).
[2] Frey, E. K., Kraut, H., and Werle, E., *Kallikrein Padutin.* (Stuttgart, Enke, 1950).
[3] Frey, E. K., and Kraut, H., *Arch. exp. Pathol. Pharmakol.*, **133**, 1 (1928).
[4] Frey, E. K., Kraut, H., and Schultz, F., *Arch. exp. Pathol. Pharmakol.*, **158**, 334 (1930).
[5] Kraut, H., Frey, E. K., and Werle, E., *Hop. Syl. Zeit. Physiol. Chem.*, **222**. 73 (1933).
[6] Werle, E., and Roden, P. von, *Biochem. Z.*, **286**, 213 (1936).
[7] Lewis, G. P., *J. Physiol.*, **140**, 285 (1958).
[8] Webster, M. E., and Pierce, J. V., *J. Pharmacol. Exp. Ther.*, **130**, 484 (1960).
[9] Lewis, G. P., *J. Physiol.*, **147**, 458 (1959).
[10] Werle, E., and Kaufman-Bretsch, B., *Hoppe-Seyler's Z. physiol. Chem.*, **319**, 52 (1960).
[11] Rocha e Silva, M., and Hamburg, U., *Proc. Twentieth Intern. Physiol. Congr.*, Brussels, 390 (1956).
[12] Rocha e Silva, M., and Hamburg, U., *Experientia*, **13**, 489 (1957).
[13] Schachter, M., *Brit. J. Pharmacol.*, **11**, 111 (1956).
[14] Armstrong, D., Jepson, J. B., Keele, C. A., and Stewart, J. W., *J. Physiol.*, **135**, 350 (1957).
[15] Andrade, S. O., Diniz, C. R., and Rocha e Silva, M., *Arch. int. Pharmacodyn.*, **95**, 100 (1953).
[16] Andrade, S. O., and Rocha e Silva, M., *Biochem. J.*, **64**, 701 (1956).
[17] Elliott, D. F., Horton, E. W., and Lewis, G. P., *Biochem. J.*, **78**, 60 (1961).
[18] Boissonnas, R. A., Guttman, St., Jaquenoud, P.-A., Konzett, H., and Sturmer, E., *Experientia*, **16**, 326 (1960).
[19] Nicolaides, E. D., and De Wald, H. A., *J. Org. Chem.* (In preparation).
[20] Elliott, D. F., Horton, E. W., and Lewis, G. P., *Biochem. Biophys. Res. Commun.*, **3**, 87 (1960).
[21] Collier, H. O. J., and Shorley, P., *Nature*, **188**, 999 (1960).
[22] Konzett, H., and Sturmer, E., *Nature*, **188**, 998 (1960).
[23] Lewis, G. P., *Nature*, **188**, 999 (1960).
[24] Collier, H. O. J., Holgate, J. A., Schachter, M., and Shorley, P. G., *Brit. J. Pharmacol.*, **15**, 290 (1960).
[25] Wanler, B. A., *J. Physiol.*, **157**, 475 (1961).
[26] Herxheimer, H., and Stresemann, E., *J. Physiol.*, **158**, 38*P* (1961).
[27] Elliott, D. F., Horton, E. W., and Lewis, G. P., *J. Physiol.*, **153**, 473 (1960).
[28] Fox, R. H., Goldsmith, R., Kidd, D. J., and Lewis, G. P., *J. Physiol.*, **157**, 589 (1961).
[29] Holdstock, D. J., Mathias, A. P., and Schachter, M., *Brit. J. Pharmacol.*, **12**, 149 (1957).
[30] Lewis, G. P., *Lectures on the Scientific Basis of Medicine*, **10** (1961).
[31] Menkin, V., *Dynamics of Inflammation* (New York: Macmillan, 1940).
[32] Menkin, V., *Newer Concepts of Inflammation* (Springfield, Ill. : Thomas, 1950).
[33] Hilton, S. M., and Lewis, G. P., *J. Physiol.*, **129**, 253 (1955).
[34] Lewis, T. H., *The Blood Vessels of the Human Skin and their Responses* (London: Shaw, 1927).
[35] Krogh, A., *The Anatomy and Physiology of Capillaries*, second ed., 238 (London, Oxford: 1929).
[36] Lecompte, J., and Troquet, J., *C.R. Soc. Biol.*, **154**, 1115 (1960).
[37] Hilton, S. M., and Lewis, G. P., *J. Physiol.*, **128**, 235 (1955).
[38] Hilton, S. M., and Lewis, G. P., *J. Physiol.*, **134**, 471 (1956).
[39] Hilton, S. M., and Lewis, G. P., *Brit. Med. Bull.*, **13**, 189 (1957).
[40] Fox, R. H., and Hilton, S. M., *J. Physiol.*, **142**, 219 (1958).
[41] Terroux, K. G., Sekelj, P., and Burgen, A. S. V., *Canad. J. Biochem. Physiol.*, **37**, 5 (1959).
[42] Schachter, M., *Polypeptides which affect Smooth Muscles and Blood Vessels*, edit. by M. Schachter, 232 (Pergamon Press).
[43] Lewis, G. P., *Physiol. Rev.*, **40**, 647 (1960).
[44] Bhoola, K. D., May May Yi Rose, Morley, J., and Schachter, M., *J. Physiol.* (in the press).

FOOD PROCESSING IN RELATION TO HEALTH

THE final session of Section I (Physiology and Biochemistry) of the British Association meeting held at Norwich on September 6 was devoted to "Food Processing in Relation to Health", but the four papers read did not—and indeed could not in the short time available—cover the complex nature of the problems implied by the title. Nor were the full implications of the subject raised in discussion, an omission which may indicate that the audience were aware that many of the questions they might have asked cannot, in the present state of knowledge, be answered. No contribution was included in the programme on the nutritive effects of food processing, a subject on which all too little information exists, but one on which it would have been useful, and perhaps salutary, to have had a summary. Nevertheless, some other aspects of food processing entered into the other papers, and if all are viewed together an otherwise fairly complete picture emerges. Miss D. F. Hollingsworth (London) described trends over eighty years in food consumption. Prof. John Yudkin (London) discussed dietary fat, and Dr. Magnus Pyke (Menstrie, Clackmannanshire) dietary protein in relation to health. Finally, Dr. J. R. Nicholls (London) reviewed the need for food preservation by means of additives and the precautions taken to ensure safety.

As a background to any consideration of trends in food consumption, it is necessary to study trends in food supplies. In the United Kingdom such statistics are readily available, for the years immediately before 1939 and for each year since the outbreak of war, in reports of the former Ministry of Food and the present Ministry of Agriculture, Fisheries and Food on food consumption-levels, now published annually in the *Board of Trade Journal*. Earlier estimates, which are not fully comparable with present ones, but which serve to indicate trends, were made by a committee set up by the British Association in 1880 to estimate the apportionment of the national income between different foods and services, with particular reference to foods; for the years 1909–13 by a committee of the Royal Society as a basis for studying the adequacy of national food supplies in the First World War; and for the years 1924–28, independently, by Sir Alfred Flux and Sir Albert Feavearyear. Examination of all these data shows that in eighty years the character of British food supplies has changed remarkably. In 1880, 55 per cent of the energy value of food supply was derived from grain and potatoes compared with 34 per cent immediately before the War, 43 per cent in the 'forties and 31 per cent in 1959. The marked changes in the other direction have been for proportions of the energy

value derived from animal products, which increased from 21 per cent in 1880 to 27 per cent in 1959; from fats and oils, which increased from 5 per cent in 1880 to 17 per cent in 1959; and from sugar, which increased from 13 per cent in 1880 to 17 per cent in 1959. Over a longer time, increasing sugar consumption is perhaps the most remarkable of all, consumption in Britain having increased five-fold in the past century and twenty-five times over two hundred years. Comparison of these trends with corresponding ones for the United States suggests that the proportions from fats and oils and sugar are unlikely to increase further, but that, if the standard of living continues to rise and manual work to decrease, the proportion from grain and potatoes may fall further with a corresponding increase in that from animal products. Such a general trend towards more food of animal and less of vegetable origin (with the possible exceptions of sugar, fruits and green and other non-starchy vegetables) is to be found in all affluent societies, and its desired nature has been recognized since the writing of the Proverb, "Better is a dinner of herbs where love is, than a stalled ox and hatred therewith".

The nutritional pattern of the national food supply in Britain, after war-time and post-war changes, became fairly stable by 1956. For all nutrients evaluated, the national diet in 1959 was of greater nutritional value than before the War. Its energy value was 5 per cent above the pre-war estimate and had scarcely changed since rationing ended in 1954. The most marked changes, compared with pre-war estimates, were for calcium and thiamine, which stood at 66 and 38 per cent, respectively, above the pre-war levels in 1947, and have remained almost unchanged since. About one-third of the increase in calcium came from increased milk supplies and the rest from the addition of *creta præparata* to flour. The restoration of thiamine to flour was largely responsible for the maintenance of the supply of this vitamin, and the restoration of iron and nicotinic acid to flour was the main cause of the increases in these nutrients. Animal protein and vitamin A also showed substantial increases. Increased consumption of dairy products was the main reason for the rise in animal protein. The rise in vitamin A came in almost equal amounts from milk and its products, liver, vegetables containing carotene, and margarine fortified with vitamin A, which more than offset reduced butter consumption.

Within this general trend towards a diet containing more foods of animal and less of vegetable origin there has been another, a striking trend towards the increased consumption during the past few years of the so-called 'convenience' foods, in which the manufacturer has relieved the housewife of much of the labour of preparation.

Supplies of canned meat and canned vegetables are nearly three times those of the pre-war years and well above those available in the early 'fifties. Supplies of canned fruit and home-produced canned soups were in 1959 more than twice as great as in 1953, while the supply of canned fish was three times as great. Consumption of unsweetened evaporated milk (which acts as a substitute for cream) is steadily increasing, but that of sweetened whole and skim milk is declining.

In the past five years the production of dried soups has doubled. Since 1954 there has been intense competition in the breakfast-cereal market which has included the introduction of sugared cereals. Biscuit production has doubled in the past ten years. Custard powder is becoming less popular, but sales of table jellies, ice-cream and soft drinks are increasing. 'Instant coffee' has rapidly gained ground, and 'instant tea' has appeared. Another development has been in frozen foods. In the past ten years consumption of frozen vegetables (almost entirely peas) has increased about eight-fold, and since 1953 consumption of frozen fish, not all of which is purchased in the frozen state, has quadrupled. A recent innovation is the sale of frozen partly prepared main dishes, such as chicken pie, and another even more recent is the production of dehydrated ready meals. The growth of the self-service shop and supermarket has probably led to a greater revolution in the presentation of meat products to the public than of other groceries or provisions.

According to the National Food Survey, nearly one-fifth of domestic food expenditure in Britain in 1959 was devoted to cooked and canned meats, other meat products, cooked and canned fish, quick-frozen legumes, canned vegetables, canned fruit, fruit juices, factory-produced cakes, pastries, biscuits and puddings, quick-cooking cereals, canned and dehydrated soups, and recent trends suggest that such expenditure will rise.

In considering dietary fat in relation to health, Prof. Yudkin said that under war-time and post-war rationing there were claims that fat consumption was too low and that this was responsible for dry skins, dull hair, a feeling of lassitude and an increase in the incidence of infection. The claim to-day is that we eat too much fat and this results in a variety of illnesses, the most important being obesity and coronary heart disease. His view is that there was no evidence that we suffered from inadequate consumption of fat during rationing and scarcely any evidence that we are suffering from excessive consumption to-day. The quantity of fat needed for human health is unknown.

Obesity, an important medical problem, is due to eating more food than is needed. Prof. Yudkin made two points on this: first, that our increasingly sedentary lives have decreased food needs, but many people are not eating less; and, secondly, that before the introduction of arable farming our prehistoric ancestors must have lived mainly on protein and fat, the outstanding dietary change occurring at the beginning of civilization being an increase in carbohydrate consumption. In the past two centuries an additional change has been the remarkable increase in the consumption of sugar with a reduction in other forms of carbohydrate. For these reasons he recommended that the best way to prevent or cure obesity is to eat much less carbohydrate and not to worry about fat intake.

Turning next to consideration of coronary heart disease, Prof. Yudkin said that there seems to be no doubt that coronary thrombosis has become more common in the past twenty years in the developed countries such as Great Britain, and that it is far less common in countries and communities where the diet contains little fat. In spite of increasing research into the subject, the main evidence relating fat consumption and coronary thrombosis is still epidemiological and no direct evidence linking the two has been produced. On the whole, populations with low consumption of fat have little coronary thrombosis. But these are also populations with low standards of living, whose diets differ in ways other than in their fat content, and whose lives differ in ways other than

diet. There are only one or two investigations in which the comparisons are of groups with similar diets but differing modes of life, or with different diets but similar modes of life; these investigations do not support the theory that dietary fat is the single factor causing coronary disease. The investigations which compare the increased incidence of the disease with the changing consumption of fat equally do not show a close relationship. The present evidence fits best the suggestion that coronary thrombosis is caused by a number of factors; these include heredity, lack of physical activity, smoking, mental stress and overweight. Prof. Yudkin concluded that there may well be other factors, and that one of these may be an excess or deficiency of one or more nutrients, such as fat in general or some kinds of fats in particular. His main conclusion was, however, a plea for more research.

Dr. Pyke's theme was the simple one that a proper understanding of the chemistry of food and the physiological requirements of people makes it possible to arrange that food processing shall be entirely beneficial to consumers. He illustrated his theme by a description of modern work on the assessment, in terms of amino-acids, of both the composition of proteins and human protein requirements. He spoke of early work on the improvement in health brought about by increased consumption by children of liquid milk and, more recently, of dried skim milk. This led to the modern approach in developing countries of studying the available protein foods, assessing their amino-acid content, mixing and producing a palatable product designed to meet the amino-acid needs of the children for whom it is intended. He gave as a concrete example the production of 'Incaparina' by Dr. Scrimshaw and his colleagues in Guatemala from maize, sorghum, cotton-seed, yeast, calcium carbonate, leaf meal and vitamin A. This can be made into a palatable gruel acceptable by children suffering from protein malnutrition. Its effectiveness was demonstrated by pictures of an affected child before and after treatment.

Dr. Nicholls spoke of man's need since prehistoric times to preserve and store foods so as to find the means of avoiding both glut and famine. By tradition, salt, sugar, vinegar and other materials have been used to preserve foods. In present urban life, other additions are necessary to prevent damage during transport and storage, and to produce for the housewife more attractive, more convenient foods of better keeping quality. Such additives must be of real practical value and must not impair significantly nutritive properties nor involve a health hazard. Dr. Nicholls described the ways of testing and controlling the use of additives not normally accepted as food ingredients, including the work of the Advisory Committee on Poisonous Substances used in Agriculture and Food Storage, the Food Standards Committee, various scientific bodies and the chemical and food manufacturers' laboratories.

D. F. HOLLINGSWORTH

SPEECH

A SYMPOSIUM on speech was held on September 5 during the British Association meeting in Norwich under the joint auspices of Sections I and J (Physiology and Psychology). The chair was taken by Prof. O. L. Zangwill (Cambridge), who pointed out that this was a near-centenary occasion. In 1868, the British Association met at Norwich and held a famous discussion on speech to which Broca and Hughlings Jackson were the main contributors. In addition to commemorating this occasion, it was hoped that the symposium would serve to draw together recent lines of work on problems of speech and illustrate changing conceptions in the study of aphasia.

Neurology of Speech

The discussion was opened by Sir Russell Brain (London), who began by paying tribute to Hughlings Jackson's remarkable contribution to the neurology of language. When Jackson addressed the British Association at Norwich at the early age of thirty-three he had already evolved the main ideas which were later to transform our thinking about aphasia. In particular, he saw clearly the need for fuller understanding of the physiology of speech and in this respect physiology had lagged behind anatomy. Whereas the work of Broca and his successors threw much light on the areas of the brain involved in language, it had failed to clarify its nature as a physiological activity. In Sir Russell's view, progress in the study of aphasia depends above all on our gaining more adequate insight into the physiological basis of normal speech.

Sir Russell proceeded to outline three lines of approach which might serve in some measure to bridge the gap between the anatomy and psychology of language. First, important clues might be derived from genetic study. In particular, we need to know how the infant's perception of his own voice monitors his speech production. In the adult, it is known that delayed speech 'playback' markedly dislocates the output of speech[1], and it is not extravagant to suppose that certain types of speech disorder owe their origin to derangement of aural 'feedback'. There is consequently a need for fuller analysis of the basic mechanisms involved in the acquisition and regulation of speech.

Secondly, Sir Russell laid stress on phonetic studies and their implications for the physiology of speech. Modern technical methods have made possible very precise analysis of the physical properties of speech sounds and have thrown a good deal of light on the characteristics of sound patterns on which intelligibility depends. Yet we still know little of the central mechanisms which govern the recognition of speech sounds and which enable constant meanings to be attached to what we hear. Context, it would seem, is an important consideration. Thus, what particular consonant is heard has been shown to depend in many instances on the vowel by which it is followed[2]. In the case of word-meaning, context is of even greater importance and raises in acute form the whole problem of serial

XUM

order in central nervous activity[3]. In view of these considerations, Sir Russell was concerned to stress the need for concepts which do adequate justice to the flexibility of language. In particular, he proposed that the concept of the 'schema', originally put forward by Head and Holmes[4] in regard to the appreciation of posture, might find fruitful application to the physiological organization of speech[5].

Thirdly, Sir Russell paid tribute to the growth of communications theory. He approved of the use of statistical method in the analysis of speech, and of the view that the brain is to be regarded as an instrument for the estimation of probabilities. Indeed, what he had called the 'schema' might perhaps be defined as the physiological mechanism which enables the individual to gamble on the probability that a particular sound pattern is to be invested with a particular meaning. He added that probability theory is now being successfully applied to the analysis of vocabulary and its reduction in aphasia.

Techniques in the Study of Aphasia

Prof. R. C. Oldfield (Oxford) was mainly concerned with the development of techniques for the analysis of specific derangements of speech. He pointed out that whereas the clinical examination of dysphasic patients has changed little since the time of Hughlings Jackson, there has been some advance in methods designed to study particular facets of the disorder. For example, Head's tests[6] were modelled on quantitative physiological procedures and were intended to assess particular derangements of symbolic function. Unfortunately, Head's theoretical views had not found general acceptance and his tests are nowadays seldom used *in toto*. Weisenburg and McBride[7] developed some more standardized tests of intellectual function, but in view of the variability of performance in aphasia it is doubtful whether their use has led to significant advance. In general, Prof. Oldfield saw little purpose in studying aphasic cases in all their bewildering variety; better, he counselled, stick to purely linguistic issues and concentrate research effort on a narrow front.

Among new methods of particular promise, Prof. Oldfield singled out the work of Penfield and his associates[8] on the electrical stimulation of the exposed human brain cortex. As is well known, stimulation within the so-called 'speech areas' reproduces many of the clinical symptoms of aphasia, among them speech arrest, misnaming, distortions and ellisions, and wrong use of words (paraphasia). It is noteworthy, moreover, that the use of this technique has failed to sustain the classical distinction between 'sensory' and 'motor' speech zones set up on the basis of clinical study. Prof. Oldfield also directed attention to the recent use of intracarotid injection of sodium amytal[9] as a means of establishing hemisphere dominance, that is, the hemisphere in which speech is located. As with cortical stimulation, transient and reversible dysphasia may be produced. Such methods, Prof. Oldfield suggested, have obvious possibilities for the experimental analysis of disordered speech.

An account of some psychological work on nominal defects and related problems was then given. As Prof. Oldfield pointed out, naming an object involves more than the mere association between symbol and referent, and disorders of naming cannot be viewed in terms of the failure of specific associative links. It is necessary, for example, to consider redundancies within the total system within which we search in seeking a particular name. More generally, Prof. Oldfield urged that statistical study of the products of disordered speech is well worth undertaking, and its arduousness is nowadays much reduced by the availability of tape recorders and electronic computers. Such study, Prof. Oldfield suggested, might well model itself on the methods employed by Yule[10] and Zipf[11] in the statistical study of normal vocabulary. Preliminary examples of the application of quantitative methods to aphasia were described[12].

In conclusion, Prof. Oldfield once again laid emphasis on the need to select limited problems and to develop techniques appropriate to their study. Although clinical issues are often dramatic, it is not always the most interesting problems that lend themselves to scientific attack.

Why we have Two Ears

The third speaker was Prof. Colin Cherry (London), who dealt with problems in the general field of speech perception. He began by directing attention to axial symmetry in bodily structure and its significance for biological adaptation. In particular, the possession of two ears is important not only for directional listening but also for effecting maximal separation of sound sources and hence optimal discrimination of speech in a noisy environment. Prof. Cherry was therefore particularly concerned to determine the features which enable the brain to infer 'what goes with what' and thereby to create unified and meaningful auditory percepts (*Gestalten*).

An important light is thrown on speech perception by consideration of the facts of binaural fusion. In the case of tones of high frequency, the half wavelength of sound is less than the inter-aural separation, and here fusion appears to depend on an 'averaging process' which extracts the fluctuating envelopes of the sounds and rejects the high-frequency steady tones[13]. This may be expected greatly to extend the range of frequencies from which stable *Gestalten* can be built. Prof. Cherry further laid stress on the role of head rotation in producing time-differences at the two ears and thereby facilitating the discrimination of speech. As he rightly pointed out, the sense of hearing appears to be closely related to the sense of balance, and posture and head adjustments are clearly of great importance in exploring the acoustic world.

Prof. Cherry then touched briefly on the relations between hearing and speech. As he has shown, an individual can listen to and repeat a spoken message virtually concurrently ('shadowing') and it is noteworthy that even stammerers can often perform this task without appreciable handicap[14]. This might suggest not only that speech and hearing form a single integrated activity but also that impediments of speech may be due to faulty aural monitoring rather than to executive disability. Indeed, such findings do much to reinforce Sir Russell Brain's plea that greater attention should be devoted to the 'feedback' mechanism in the regulation of normal speech.

The problem of binaural speech perception was next considered, with special reference to dichotic listening. Some years ago, it was shown that if two different messages are fed simultaneously through

headphones, one to each ear, the subject can 'shadow' either message at will but is rendered almost totally unaware of what is reaching the 'neglected' ear[15]. In real life, however, we seldom listen to competing sources of information in so simple a manner. More commonly, the listener is assailed by a barrage of voices which have different mutual time-relations at the two ears and various other small differences. In separating them, it is necessary to assume a kind of inductive inference based in part on past experience. Can this be studied ?

As a first step, Prof. Cherry described an experiment still in progress in which the subject wears headphones and listens to a message recorded on a special tape recorder. This enables various time intervals to be introduced between right and left headphones and thus causes the fused sound image to move from side to side in the listener's head. A second voice which may likewise be caused to move is then introduced. With the first voice set at a given position, the second is moved to right or left of it randomly by various amounts, and at each setting the subject guesses whether the shifted voice is to the right or left of the fixed voice. Although the statistics of the guesses show some measure of uncertainty, the task can in general be performed. On the other hand, if sounds provided by random noise generators are substituted for the voices, it becomes almost impossible to accomplish ; indeed the listener hears two noises as a single fused sound[16]. The next step in the experiment is to proceed either by removing clues from the voices (for example, by removing larynx pitches) or by adding clues to the random noise sources (for example, by introducing a sharp resonance) ; in each case the measure of uncertainty shows the extent to which the clues in question are utilized. Although the results are not yet complete, Prof. Cherry held out good hopes that the technique would prove powerful in enabling us to specify "what there is about human voices that enables them to be separated so efficiently".

It will be seen, then, that Prof. Cherry was concerned to isolate the factors involved in the segregation of complex auditory experiences. This analysis, he submitted, must be set out in the mathematics of statistical inference. It must be supposed that the brain is able to carry out a kind of 'correlational analysis' which enables it to identify certain invariant clues under an infinite variety of uncertain transformations. These clues are to be regarded as statistical invariants, and correlations (or hierarchies of correlations) are required to assess them. So far, he had been concerned only with first-order, short-term correlation functions[17], which allow a start to be made in describing the logic of certain perceptual operations. These computations, he suggested, which involve operations of short-term storage, coincidence detection and averaging of sensory data, may well be 'natural' to the central nervous system. The ultimate test of such an approach is, however, physiological, and Prof. Cherry concluded by asking physiologists whether, when considering the brain as a computer, it had been looked at from this point of view.

In his closing remarks, Prof. O. L. Zangwill contrasted the approach to speech evidenced by the contributors to this symposium with that of their predecessors ninety-three years ago.

First, he said, it is plain that much less weight is attached nowadays to consideration of structure. Unlike Broca, Sir Russell Brain had brought along no colourful charts to demonstrate the cerebral seat of speech and evidently did not regard anatomical issues as of prime importance. Although the problem of cerebral localization of course remains, it now seems doubtful whether clinico-pathological study alone can throw important light on the mechanism of speech. Indeed, neurologists appear to be turning increasingly to neurophysiology, experimental psychology and communications theory to help in the elucidation of clinical phenomena. In this field at least, pathology has proved itself a disappointing guide to our understanding of normal function.

Secondly, Prof. Zangwill directed attention to some striking changes in our general conception of the speech processes. In Broca's day, it was customary to break down speech into a multiplicity of elements, sensory and motor, and to suppose that each of these possessed determinate cerebral localization. Even Hughlings Jackson had not been wholly free from this tendency to over-simplify. To-day, it would seem, our approach is decidedly anti-analytical ; no one had spoken of sensations, images or related products of introspective abstraction. Indeed, the emphasis had been almost wholly on higher-order organization and on the brain mechanisms conceived to underlie it. This had been particularly well brought out by Prof. Cherry in his valuable studies of binaural integration and the perception of speech.

Lastly, Prof. Zangwill commented on the astonishing extent to which statistical thinking now dominates our approach to problems of language. Statistics were unknown to Hughlings Jackson, but it would appear that his successors can ill afford to disregard them. No longer can speech be envisaged in terms of reflexes and habits of an essentially particulate nature, and no longer can disorders of speech be viewed in terms of the loss of particular reactions or classes of reaction. As Prof. Oldfield so well showed, analysis of the products of disordered speech cannot be satisfactorily undertaken without recourse to considerations of probability theory. Although information theory may give difficulty to the non-mathematical, clearly it must be accepted if our knowledge of speech is to progress.

[1] Lee, B. S., *J. Acoust. Soc. Amer.*, **22**, 824 (1950).
[2] Liberman, A. M., Delattre, P., and Cooper, F. S., *Amer. J. Psychol.*, **65**, 497 (1952).
[3] Lashley, K. S., in *Cerebral Mechanisms in Behaviour*, edit. by Jeffress, L. A. (Wiley, New York, 1951).
[4] Head, H., and Holmes, G., *Brain*, **34**, 102 (1911–12).
[5] Brain, R., *Brain*, **84**, 145 (1961).
[6] Head, H., *Aphasia and Kindred Disorders of Speech* (Camb. Univ. Press, London, 1926).
[7] Weisenburg, T., and McBride, K. E., *Aphasia: A Clinical and Psychological Study* (Commonwealth Fund, New York, 1935).
[8] Penfield, W., and Roberts, L., *Speech and Brain Mechanisms* (Univ. Press, Princeton, 1959).
[9] Rasmussen, T., and Wada, J., *J. Neurosurg.*, **17**, 266 (1960).
[10] Yule, G. Udny, *The Statistical Study of Literary Vocabulary* (Camb. Univ. Press, 1944).
[11] Zipf, G. K., *The Psycho-biology of Language* (Houghton Mifflin, Boston, 1935).
[12] Elvin, M., and Oldfield, R. C., *J. Neurol. Neurosurg. Psychiat.*, **14** 118 (1951).
[13] Leakey, D. M., Sayers, B. McA., and Cherry, E. C., *J. Acoust. Soc. Amer.*, **30**, 222 (1958).
[14] Cherry, E. C., Sayers, B. McA., and Marland, M. P., *J. Psychosom. Res.*, **1**, 233 (1956).
[15] Cherry, E. C., *J. Acoust. Soc. Amer.*, **25**, 975 (1953).
[16] Cherry, E. C., and Bowles, J. A., *J. Acoust. Soc. Amer.*, **32**, 884 (1960).
[17] Sayers, B. McA., and Cherry, E. C., *J. Acoust. Soc. Amer.*, **29**, 973 (1957).

OBITUARIES

Prof. H. V. A. Briscoe

Henry Vincent Aird Briscoe died in London after a short illness on September 24, 1961, his seventy-third birthday. He was professor of inorganic chemistry in the University of London at the Imperial College of Science and Technology during 1932–54 and thereafter professor emeritus and Fellow of the Imperial College. Briscoe was educated first at the City of London School and then the Royal College of Science, which at the time was on the point of being incorporated within the framework of the Imperial College. He graduated with honours in chemistry and then first assisted Sir Edward Thorpe in a revision of his *Dictionary*. Although he was still a junior member of the teaching staff of the Imperial College, during the First World War he was engaged in responsible and secret work (which similarly occupied much of his time during the Second World War) and later took charge of the Organic Chemistry Department at Sir John Cass College before being appointed in 1921 to the chair of inorganic and physical chemistry at Armstrong College (now King's College), Newcastle upon Tyne. He became head of the Chemistry Department in 1925 and later served as dean of the Faculty of Science in the University of Durham. Six years following his return to the Imperial College, on the retirement of Prof. J. C. Philip, he became director of the Laboratories of Inorganic and Physical Chemistry, a position he held until his retirement.

In addition to his academic positions, Briscoe had many outside interests: among other things he was the first director of the Northern Coke Research Committee's laboratory, president of the Research Association of British Paint, Colour and Varnish Manufacturers, visitor and manager of the Royal Institution of Great Britain and Ireland, vice-president of the Royal Institute of Chemistry, as well as being a member of Council and officer of the Chemical Society, and chairman of a local section of the Society of Chemical Industry. After the Second World War he was also greatly concerned with the improvement of the supply of laboratory technicians in university work and was chairman of the Imperial College Technician Training Committee, of the similar University of London Committee and of the National Joint Committee on the Recruitment and Training of Science Laboratory Technicians.

Briscoe's scientific publications cover a wide range of interests. Although his first publication in 1912 with P. W. Robertson was on the migration of the *para*-halogen atom in phenols, he afterwards moved into the inorganic and physical area. Among his earlier works were precise atomic weight determinations of vanadium, tin, thallium, boron and bromine. He considered himself a disciple of T. W. Richards and was similarly devoted to rigorous purification and the development of new methods, such as those for handling volatile hydrolysable halides. He was also aware of the possibility of the variation in isotopic ratios of materials of different geological origin, and for his studies on boron developed a flotation method for the measurement of comparative densities of boron trichloride which gave values to within about $\pm$ 2 $\times$ 10^{-6} unit. This density method, which was typical of Briscoe's experimental skills and ingenuity, was also applied to examine heavy water and he was among the first in Great Britain to be concerned with the use of deuterium and later oxygen-18 as tracers in exchange reactions. Briscoe also made contributions to the chemistry of rhenium oxides, halides and sulphides, and of selenium.

Throughout his career, Briscoe was much concerned with more applied aspects of chemistry and made many contributions to the knowledge of coke, paints and drying oils, on which he was an authority, purity of water, insect pests in foodstuffs, etc. During the 1930's he became very interested in the silicosis problem and hazardous industrial dusts and devised methods for collection and analysis of dust samples. For this work he was awarded the Consolidated Goldfields of South Africa Medal by the Institution of Mining and Metallurgy. Inspired by a lecture given by Langmuir at the College, and anticipating current interest by many years, he also worked with Sebba on the reduction of evaporation from water surfaces through the use of monolayers of cetyl alcohol.

Briscoe will be remembered by his colleagues and students for his approachability and kindly manner. He is survived by his wife and a son and daughter—both members of the medical profession.

G. Wilkinson

Prof. Carl Hermann

Prof. Carl Hermann, director of the Crystallographic Institute at the University of Marburg, died unexpectedly in his sleep on September 12 at the age of sixty-three. He was a pupil of Max Born and a fellow research student with Werner Heisenberg, at Göttingen. Later he became a lecturer at Stuttgart, where he and Paul Ewald initiated the *Strukturberichte* which (with its successor *Structure Reports*) gives a detailed abstract of every known crystal structure determination, and which is a valued work of reference in every modern scientific library. He also developed, with Prof. C. Mauguin, a systematic nomenclature (now known as the Hermann–Mauguin notation) for the 230 space groups, from which the symmetry can be derived at a glance; and he travelled widely in the early 1930's, gathering material for the two volumes of the *Internationale Tabellen zur Bestimmung von Kristallstrukturen*.

Hermann and his wife Eva were guests in my home at the time when the National Socialist party doubled its strength in the General Election of June 1932, which later brought Hitler to power; and I can well remember their dismay, for they realized even then what would follow. Later, Carl Hermann refused to conform to the political conditions imposed on academic staff and he took a position as physicist at the I.G. Farbenwerke at Ludwigshafen, where he continued crystallographic research of high quality on the nature of chemical bonds and on symmetry in n-dimensional space. He and his wife were imprisoned throughout much of the Second World War for their increasing activities in hiding and helping innumerable Jews to escape from the terrors of Nazi persecution. Only his eminence as a scientist

and the intervention of his friends saved him from the carrying out of a death sentence; but these years left their mark on him.

In 1947 he was appointed to the new chair of crystallography in the University of Marburg and he threw himself with great energy into the teaching of the subject and into the building-up of an active research school. But he also gave much of his time to the work of the Society of Friends, of which he and his wife were members, and to efforts to promote international understanding. His presence at conferences was the more valued because of his great gift for languages; if a new language interested him, he learnt it.

The statement put out by the Rector and Senate of the University of Marburg says of him: "Carl Hermann was a man of absolute integrity, and of kindness to others which had no limit. For many years he was a member of the Disciplinary Court of the University and it was always he who tried to understand and to excuse the defendant . . .".

He is not likely to be forgotten, for he has left many friends. KATHLEEN LONSDALE

Prof. E. Taylor Jones

THE death on September 29 of Prof. Edward Taylor Jones at the age of eighty-eight will have been noted with deep regret by the many who benefited from his unique qualities both as a teacher and research supervisor. His own education followed a pattern that was familiar at the turn of the century. His studies at University College, Bangor, and the University of Berlin were followed by a time as professor of physics at Bangor and a long period as professor of natural philosophy in the University of Glasgow, a period prolonged by the advent of war in 1939. His interests throughout his life extended over a wide field of physics and he seemed, in fact, to be equally interested in physics and in electrical engineering. Thus, his earlier researches were in the classical field of electromagnetism with special reference to the mechanism of oscillatory devices such as the singing arc, the induction coil, the Tesla coil, and the magneto.

Research into the behaviour of the induction coil may well have led to his very early attack on a variety of problems in electron diffraction. He was greatly excited by the elegant de Broglie approach to wave mechanics and soon followed the lead given by G. P. Thomson at the University of Aberdeen in his famous work on electron diffraction. This was a notable switch in interest and could have resulted in a greater volume of original work but for the limitations of means and research students of that time. Nevertheless, on average he supervised the work of some five research students throughout his active career. I myself realized, as one of his research students, that Taylor Jones made many fruitful suggestions while never stultifying the initiative of members of his team. He preferred to play the part of adviser and in this he gave freely of his store of knowledge.

He will doubtless be remembered by most as an inspired teacher of the very large ordinary classes. These seemed to fascinate him. He took great pains to make his courses lively and even at times hilarious, obviously believing that above all interest must be generated. His extremely expert clowning in some of his demonstrations made it inconceivable that an understanding of the fundamentals should be beyond any student. In spite of this unique approach to his ordinary classes there was considerable discipline in his handling of classes of more than two hundred students. With the advanced classes he was content to follow more orthodox patterns of instruction. He must have roused within many a life-long interest in physics by his vivid proof that science could be fun.

Prof. Taylor Jones had many publications to his credit and the list of these shows his versatility and widely ranging interests. Physics made startling advances throughout his life and he was excited by many of them. He did not confine his research interests closely and he never looked with favour on team-work. In this respect he belonged with the individualists of his earlier period. Those who knew him as a friend realized how fully he enjoyed his life spent in physical science. S. C. CURRAN

Dr. Madeline Kerr

IT was with deep regret that the colleagues and friends of Dr. Madeline Kerr learned of her death on August 22, a few months after she had resigned from her post of senior lecturer in social science in the University of Liverpool because of ill-health.

Dr. Kerr was primarily a social psychologist, but the richness and variety of her interests in the cultural process took her work into the sociological and anthropological fields. Dr. Kerr graduated at Manchester and, after working in the Psychological Service of the Liverpool Education Committee, returned to take up the Knight fellowship in psychological medicine. At Manchester she was influenced by Prof. T. H. Pear and retained an interest in the interaction of social status and personality traits. From Manchester she went to London, where she took her doctorate and worked with Prof. Lancelot Hogben. She then joined Prof. Susan Stebbing to teach psychology in the Department of Philosophy at Bedford College. She was a great admirer of Susan Stebbing and this was reflected in the logical austerity of her seminar teaching.

In 1947 Dr. Kerr was given leave of absence to act as social psychologist to the West Indian Social Survey, where she organized a programme of psychological testing. In the West Indies, Dr. Kerr found an environment in which her interests in the graphic and plastic arts and in music combined with her scientific interests to make her immediately acceptable to the subjects of her study, and she made lasting friendships with members of all classes in West Indian society from simple peasants to members of the Government. Some of the findings of her work were published in *Personality and Conflict in Jamaica* (1952).

On her return to England it was natural that she should join Prof. T. S. Simey, who had also worked in the West Indies, in the Department of Social Science of the University of Liverpool. Here she continued her intensive investigation on families, and in 1958 published *The People of Ship Street*, which described the existence of a matriarchal society in a working-class area of Liverpool.

In Liverpool she devoted most of her energies and her great enthusiasm to her students, for whom nothing was too much trouble and with whom she made close and enduring friendships. She will be greatly missed.

D. CHAPMAN

XUM

NEWS and VIEWS

Royal Society: Medal Awards

The following awards of medals have been made by the President and the Council of the Royal Society: *Copley Medal* to Sir Hans Krebs, Whitley professor of biochemistry in the University of Oxford, for his distinguished contributions to biochemistry, in particular his work on the ornithine, tricarboxylic acid and glyoxylate cycles; *Davy Medal* to Prof. D. H. R. Barton, professor of organic chemistry in the Imperial College of Science and Technology, University of London, for his distinguished researches in organic chemistry, particularly on the structure and stereochemistry of natural products of the terpene and steroid series; and the analysis of the conformation of cyclic structures; *Sylvester Medal* to Prof. P. Hall, Sadleirian professor of pure mathematics in the University of Cambridge, for his distinguished researches in algebra; *Hughes Medal* to Prof. A. H. Cottrell, Goldsmiths' professor of metallurgy in the University of Cambridge, for his distinguished work on the physical properties of metals, particularly in relation to mechanical deformation and to the effects of irradiation.

David Sarnoff Award in Electronics : Dr. C. H. Townes

Dr. Charles H. Townes, a leading American physicist and provost of the Massachusetts Institute of Technology, has been presented with the David Sarnoff Award in Electronics of the American Institute of Electrical Engineers. The award cited Dr. Townes for research in physics leading to major advances in communication technology. He is well known for his work on the theory and application of masers, on which he holds the fundamental patent. Dr. Townes was appointed provost of the Massachusetts Institute of Technology earlier this year. He was a member of the technical staff of Bell Telephone Laboratories during 1939–47, then joined the faculty of Columbia University as associate professor of physics. He was appointed professor in 1950, served as executive director of the Columbia Radiation Laboratory during 1950–52 and as a chairman of the Physics Department during 1952–55. Dr. Townes's awards include the Morris Liebman Memorial Prize of the U.S. Institute of Radio Engineers (1958), the Stuart Ballantine Medal of the Franklin Institute (1959), the Comstock Award of the U.S. National Academy of Science (1959) and the Rumford Premium of the American Academy of Arts and Sciences (1961).

National Physical Laboratory : Dr. J. V. Dunworth, C.B.E.

Dr. J. V. Dunworth has been appointed deputy director of the National Physical Laboratory (Department of Scientific and Industrial Research) in succession to Dr. G. Macfarlane, who early next year will take up his recent appointment of director of the Royal Radar Establishment, Malvern (*Nature*, 192, 18; 1961). Dr. Dunworth is at present deputy director of the Atomic Energy Establishment, Winfrith. He is forty-four years of age and was born in Manchester and educated at Manchester Grammar School and the University of Cambridge. Shortly before the outbreak of war in 1939 he was invited to become a member of Sir John Cockcroft's team investigating problems in the use of radar. He continued in this work until 1944, working on 150-cm. and 10-cm. radar development, mainly at the Radar Research Establishments at Christchurch and Malvern. Late in 1944 he went to Canada with Sir John Cockcroft to work with the National Research Council on atomic energy development. Soon after the end of the War he returned to Cambridge to take up his fellowship at Trinity College and was appointed a demonstrator in physics in the Cavendish Laboratory. Later, in 1946, he rejoined Sir John Cockcroft at the Atomic Energy Research Establishment, Harwell.

The Botanical Survey of India: Prof. H. Santapau

For a long time the Botanical Survey of India remained in hibernation and the superintendent of the Sibpur Botanical Garden was also the officer-in-charge of the Botanical Survey with its headquarters in the Indian Museum at Calcutta. About eight years ago the Survey was re-organized and now has a new headquarters at Calcutta together with a number of regional offices in different parts of the country. Dr. J. C. Sen Gupta, who was chief botanist for the past several years, retired last summer and was succeeded a few months ago by Father H. Santapau, for many years professor of botany at St. Xavier's College, Bombay. Father Santapau was born in Spain in 1903. He went to India in 1928 and has ever since been intimately associated with educational institutions in India. Although his original education was in Barcelona in Spain, he later studied in the Royal College of Sciences and Imperial College of Science and Technology, London, from which he obtained the diplomas of A.R.C.S. and D.I.C. and the B.Sc. honours and Ph.D. degrees of the University of London. In 1947 he was elected Fellow of the Linnean Society of London and in 1953 a Fellow of the National Institute of Sciences of India. Father Santapau is the author of a large number of research papers and books dealing with the results of his explorations in parts of the Western Ghats (especially Mahabaleshwar) and the Dangs Forest in Saurashtra. A few years ago he wrote a comprehensive *Flora of Khandala* (a second edition of which has now been published) and in 1958 the *Flora of Purandhar*. Among his other publications special mention may be made of one on the *Acanthaceae of Bombay* and another dealing with a *History of Taxonomic Researches in India on the Angiosperms*.

Twenty-five Years of Television

During the first week of November, the British Broadcasting Corporation celebrated the inauguration of the world's first high-definition television service—on November 2, 1936 (*Nature*, 138, 394, 793; 1936). At the beginning two systems of transmission, the Baird and the Marconi-EMI, were used in alternate weeks to judge under practical conditions which was the better. After three months of this experimental service, and following the recommendation of H.M. Postmaster-General's Television Advisory Committee, the transmissions were confined to the Marconi-EMI system on a standard basis of interlaced scanning at

405 lines per picture, and a picture frequency of 25 per sec. It was in this fashion that what is generally conceded to be the best public television service in the world opened well in advance of the United States, where the first public television transmissions did not start until April 1939.

It is a tribute to the excellent pioneer work of the engineers and scientists who formulated the technical standards adopted by the Government in 1937 to note that these have remained unchanged in Britain for twenty-five years. Associated with the celebrations of this silver jubilee was a dinner given by the British Broadcasting Corporation at Grocers' Hall, on November 7, presided over by Sir Arthur ffordo, chairman of the B.B.C. The Prime Minister proposed the toast of the service and paid tribute to the pioneer work of all those concerned not only with the technical achievement but also with the foundation of the new techniques of programme provision and presentation, for both informative and entertainment purposes. Many of those responsible for these developments were present on this occasion, and they and their successors must have been encouraged to work towards the further developments which will undoubtedly occur in the next quarter of a century.

Milk Powder

Even the richest cows' milk contains some 86 per cent of water, and it has long been realized that if milk could be dried satisfactorily at a moderate cost to produce a product with practically the same nutritive value as the raw milk from which it came, the resulting powder would have many important uses. It is not surprising to find, therefore, that the manufacture and use of dried milk are greatly increasing in many parts of the world, and that even in Britain the amount now made is three times as much as it was before the Second World War. The Government's Milk and Milk Products Technical Advisory Committee, realizing that many potential users of milk powder might not know of the merits and value of the modern product, or about the different types that are available, sponsored the preparation of a booklet by its Milk Products Sub-Committee (Ministry of Agriculture, Fisheries and Food; Department of Agriculture and Fisheries for Scotland; Department of Health for Scotland. *Milk Powder*. Pp. v+18. (London: H.M.S.O., 1961.) 1*s*. 3*d*.).

The booklet succeeds in giving a most valuable account of the subject, and is divided into two parts, the first of which deals with the manufacture of milk powders, their composition, bacteriology, bulk reconstitution to form liquid milk and their nutritive value. The first part contains also a section on the physical and chemical properties of milk powders in which important properties such as particle size and shape, bulk density, flow properties and solubility and dispersibility in water are discussed. The second part gives an account of the commercial uses of dried milks in baking and in the manufacture of margarine, chocolate, manufactured meat products, soups and ice-cream. It deals also with the use of milk powder in canteens and institutions, and with its value in feeding-stuffs for certain classes of farm livestock such as very young calves, chicks and turkey poults. The booklet ends with a list of eighteen references from which further details can be obtained. This small but most informative booklet will long remain a standard work of reference on a subject which is bound to increase in importance as food science and food technology develop with increasing application of these subjects to world food problems.

Royal Ontario Museum : New Mineral and Gem Gallery

A new mineral and gem gallery at the Royal Ontario Museum, University of Toronto, has been financed by a 75,000 dollar grant from the International Nickel Company of Canada, Ltd. The exhibit of minerals on show in the Gallery will include material from the Leonard Collection of gemstones, the Andre Dorfman Collection of minerals and the MacIntyre–Porcupine Collection of gold ore and nuggets. The mineralogy collections now at the Royal Ontario Museum were begun at Victoria College, Cobourg, at the end of the nineteenth century, at the Provincial Museum, Toronto, and with the purchase by the University of Toronto of one of the collections assembled by the late Dr. W. F. Ferrier. In 1912, when the Royal Ontario Museum was established, these collections were brought together.

The Cotton, Silk and Man-made Fibres Research Association

Speaking at the annual general meeting of the Cotton, Silk and Man-made Fibres Research Association on October 12, the director, Dr. D. W. Hill, said that the work of the Association was now organized in fourteen departments, of which five were newly constituted, and the research programme was controlled by a new Research Committee served by nine sub-committees, including two new ones. All the 110 members of staff declared redundant on the merging of the two research associations had found satisfactory positions elsewhere. The report of the Council, presented at the annual meeting, in describing research and development during the year refers to the application of the principle of the Shirley 'automatic size box' to the feed-box of a mangle and to progress in overcoming difficulties associated with a self-brushing device for laying the fibres in warps during sizing. It also refers to work on heat transmission through assemblies of aluminized fabrics and cotton blankets with the view of providing information on which can be based the design of clothing for protection under conditions more exacting than those met in ordinary life. Special attention has been paid to the development of a cold-pad process for bleaching cotton–acetate unions without hydrolysing or delustring the acetate, and considerable progress has been made in work on the flow of liquids in contact with or through textiles. Work of package dyeing has directed attention to methods of achieving complete wetting-out of the package.

Men and their Jobs

For his inaugural lecture delivered at Birkbeck College, University of London, on October 16, Prof. Alec Rodger took as his title "Occupational Versatility and Planned Procrastination". Prof. Rodger indicated that existing surveys to discover the requirements of man-power are usually inaccurate, and that, despite what is being done to prevent occupational waste, there is much human wastage for which remedial work has to be done. At present, 10,000 cases pass through the industrial rehabilitation units of the Ministry of Labour each year. Prof. Rodger suggested that facilities which are at present intended mainly for the disabled or chronically employed

XUM

should be extended to other sections of the community. With ten more occupational psychologists in the Ministry of Labour, considerable help could be given in providing better guidance for both adults and young people. To avoid real human waste, however, the training people received for jobs should be given the widest possible base, and specialization should be postponed as long as possible, until the individual and his advisers are reasonably sure he is in the right occupation or profession.

Nature Reserves

The forty-fourth annual report of the Society for the Promotion of Nature Reserves for the year ending March 31 is largely made up of accounts of the activities at the fourteen reserves which come under the jurisdiction of the Society (Pp. 44+2 plates. London: Society for the Promotion of Nature Reserves, British Museum (Natural History), 1961. 10s.). At Woodwalton Fen, Huntingdonshire, a study of the breeding biology of the large copper butterfly was continued and the eggs and larvæ on more than a thousand food-plants are being counted at regular intervals. Observations will be carried out on the preference shown by the butterflies when egg-laying for docks growing in the open fen and those growing in the dykeside. Suggestions for introducing the butterfly on an experimental basis to Blackmoor Copse Nature Reserve, Wiltshire, have been considered, but it has now been decided to postpone a decision until further observations have been made at Woodwalton Fen. A spider new to the British list, *Asagena phalerata*, was recorded at Linwood Warren Nature Reserve, while at Calf of Man, Isle of Man, a red-breasted flycatcher (the second Manx record) was captured in June, and Manx shearwaters were taken on land during the summer. The Manx shearwater has not been seen on the island for nearly two hundred years. At Belfairs Great Wood, Essex, the reed mace grass veneer (*Crambus paludellus* Hübner) has now occurred two years running in a garden abutting on the Reserve, and is probably breeding on reed mace in one of the small ponds.

Electrotechnology in Australia

In the annual report of the Division of Electrotechnology of the Australian Commonwealth Scientific and Industrial Research Organization for the year ended June 30, 1960, reference is made to the three-weeks visit paid by Prof. H. Fröhlich, professor of theoretical physics in the University of Liverpool, to Australia at the invitation of the Division. The object of the visit was to discuss and advise on the various aspects of the research work of the Dielectrics Section of the Division, which is concerned with the study of the alkali halides, aliphatic long-chain compounds—particularly the growth of large single crystals and the production of pure compounds; the hydrates of calcium sulphate; and the theories of dielectric loss and of superconductivity. The Division is responsible for the maintenance of the Commonwealth standards of measurement of electrical and magnetic quantities, and its activities are dealt with separately in the annual report under the headings of direct current, power frequency, audio- and radio-frequency, and magnetics. With the completion of the calculable capacitor, considerable progress has been made with necessary additional equipment and techniques for the absolute determination of the ohm. The screening efficiency of screened rooms with especial emphasis on the microwave region of the spectrum has been investigated, and work on the probability problems associated with various random processes has been carried out experimentally by means of the probability distribution analyser designed and constructed in the Division. The report concludes with a list of the publications by members of the staff, and their names and positions as at June 30, 1960.

Solid Propellant Test Rig

Messrs. P. A. Hilton and Co., engineers, of Radlett, Hertfordshire, announce that they have completed the development of a solid propellant rocket motor test rig for use in universities and technical colleges. The rig consists of a stainless steel rocket motor suspended on a swinging parallel motion linkage which pushes against a cantilever spring beam. Movement under load is transmitted to a pen recorder indicating thrust, on paper moved at constant speed by a synchronous electric motor. The area of the diagram produced is related by the calibration to the total impulse. Chamber pressure is transmitted by flexible pipe to a bourdon tube which in turn operates a recorder pen running parallel with the thrust pen. The fuel used is a form of cordite supplied in cartridge form giving a specific impulse of about 180 sec. and requiring no more elaborate storage facilities than are accorded to shotgun ammunition. The motor is equipped with a blow-out diaphragm to diminish explosion risks. The rig permits measurement of thrust, total impulse, specific impulse and burning time, and it is possible to study the effect of chamber pressure and nozzle design on these quantities by substituting nozzles of different diameters and profiles.

The rig is of simple design, of which the recorder is the weakest link, since it is not certain that the relation between diagram area and total impulse is strictly linear and since the mechanism for applying tension to the paper is rather crude. The rig, however, should prove valuable for teaching and demonstrating in engineering departments wherever an interest in rocket motors and combustion exists, and it has been constructed on a scale such that departments with quite limited facilities can use it. The rig is a welcome addition to the very limited range of standard teaching apparatus available in the sphere of gas dynamics and combustion. Further details can be obtained from the manufacturers.

Summer School in Analytical Chemistry

The fifth triennial summer school in analytical chemistry, organized by the Royal Institute of Chemistry with the participation of the Society for Analytical Chemistry, will be held in the Manchester College of Science and Technology during September 9–15, 1962. The School will consist of four separate, but concurrent, courses: *Course* 1, "Physical Methods of Organic Chemistry", under the direction of Dr. D. W. Mathieson, reader in pharmaceutical chemistry, School of Pharmacy, London; *Course* 2, "Recent Developments in Inorganic Analysis", under the direction of Mr. W. T. Elwell, chief analyst, Imperial Chemical Industries, Ltd., Metals Division, Witton, Birmingham; *Course* 3, "The Determination of Toxic Substances in the Air and in Effluents", under the direction of Mr. H. E. Stagg, chief analyst, Imperial Chemical Industries, Ltd., Dyestuffs Division, Blackley, Manchester; *Course* 4, "Newer Instrumental Techniques", under the direction of Dr. V. S. Griffiths, reader in spectroscopy, Battersea College of Techno-

logy, London. Further information can be obtained from the Education Officer, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

Theoretical Chemistry Summer School at Oxford

Once again Prof. C. A. Coulson and the Delegacy for Extra-Mural Studies of the University of Oxford are organizing a summer school in theoretical chemistry. The school is to be housed in St. Antony's College, with lectures in the Mathematical Institute. Further information may be obtained from the Secretary, Delegacy for Extra-Mural Studies, Rewley House, Wellington Square, Oxford. The dates of the School are September 8–22, 1962; accommodation is strictly limited and the closing date for applications is April 30, 1962.

British Institution of Radio Engineers: Awards

The following awards have been made by the British Institution of Radio Engineers for outstanding papers published in the Institution's *Journal* during 1960: *Clerk Maxwell Premium*, to Dr. G. T. Wright (University of Birmingham) for his paper on "A Proposed Space-Charge-Limited Dielectric Triode"; *Heinrich Hertz Premium*, to Mr. R. A. Waldron (Baddow Research Laboratories, Marconi's Wireless Telegraph Co.) for his paper on "Features of Cylindrical Waveguides containing Gyromagnetic Media"; *A. F. Bulgin Premium*, to Dr. A. Kraus (Rohde and Schwarz, Munich) for his paper on "Reflection Coefficient Curves of Compensated Discontinuities on Coaxial Lines and the Determination of the Optimum Dimensions"; *Arthur Gay Premium*, to Mr. E. Davies (Marconi's Wireless Telegraph Co.) for his paper on "The Application of Printed Wiring to Development of Small Batch Production with Particular Reference to Television Equipment"; *Leslie McMichael Premium*, to Mr. C. H. Dix and Mr. W. E. Willshaw (Hirst Research Centre of the General Electric Co.) for their joint paper on "Microwave Valves: a Survey of Evolution, Principles of Operation and Basic Characteristics"; *Lord Rutherford Award*, to Mr. E. C. Fellows (Associated Electrical Industries Research Laboratories, Aldermaston) for his paper on "Energy Stabilization of a 4-MeV. Electrostatic Accelerator using Controlled Corona Discharge"; *Charles Babbage Award*, to Mr. E. M. Bradley (International Computers and Tabulators) for his paper on "A Computer Storage Matrix using Ferromagnetic Thin Films".

The Night Sky in December

New moon occurs on Dec. 7d. 23h. 52m. U.T. and full moon on Dec. 22d. 00h. 42m. The following conjunctions with the Moon take place: Dec. 11d. 00h., Saturn 2° S.; Dec. 11d. 14h., Jupiter 2° S.; Dec. 20d. 11h., Aldebaran 0·7° S.; Dec. 26d. 21h., Regulus 0·5° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Antares on Dec. 11d. 20h., Venus being 5° N. Mercury and Mars are too close to the Sun for observation. Venus is a morning star, but is also too close to the Sun for easy observation. Jupiter and Saturn are evening stars, setting about three hours after the Sun, but conditions for observation are not favourable. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Dec. 1d. 1h. 19·3m., σ Leo. (*D*); Dec. 1d. 2h. 13·5m., σ Leo. (*R*); Dec. 12d. 17h. 56·7 m., 45 Cap. (*D*); Dec. 18d. 19h. 46·6m., + 11° 445 (*D*); Dec. 20d. 4h. 25·0m., γ Tau. (*D*); Dec. 23d. 23h. 30·4 m., 81 Gem. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Geminid meteors are active during December 9–14, maximum activity being on December 13. The radiant is at R.A. 7h. 28m., Dec. + 32°, and conditions for observation are very favourable. The Ursid meteors are active on December 20–22, but conditions for observation are unfavourable. The winter solstice occurs on December 22d. 03h.

Announcements

Mr. R. Blyth has been appointed editor of *The Pharmaceutical Journal*. He has been responsible for the *Journal* since June this year. He joined the *Journal* as assistant editor in 1957 from the same post on *The Chemist and Druggist*.

The Royal Agricultural Society of England offers annually a medal together with an award of one hundred guineas for research work of outstanding merit carried out in the United Kingdom which has proved, or is likely to prove, of benefit to agriculture. Recommendations from heads of university departments or research organizations for the next award must be submitted to the Secretary, Royal Agricultural Society of England, 35 Belgrave Square, London, S.W.1, not later than January 31, 1962.

A symposium on "The Role of Science in the Development of Natural Resources with particular reference to Iran, Pakistan and Turkey" is to be held in Lahore during January 8–13. The symposium is sponsored by the Scientific Council of the Central Treaty Organization.

The sixty-eighth annual meeting of the American Mathematical Society will be held in Cincinnati, Ohio, during January 22–25, in conjunction with the annual meeting of the Mathematical Association of America. Further information can be obtained from the associate secretary, J. W. T. Youngs, American Mathematical Society, 190 Hope Street, Providence 6, Rhode Island.

The Department of Municipal Engineering of the Manchester College of Science and Technology is organizing a short course of lectures on "Problems of Radioactivity at Water and Sewage Works", which will be held in the College during January 11–12. Further information can be obtained from the Registrar, Manchester College of Science and Technology, Manchester 1.

The International Atomic Energy Agency is organizing a conference on "New Techniques in Biology and Medicine" at Mexico City during November 21–December 1. Special attention will be directed to the use of isotopes in these fields. Further information can be obtained from the International Atomic Energy Agency, Vienna.

The Chemical Research Committee of the Indian Council of Scientific and Industrial Research is organizing a symposium on "Carbohydrates, Cellulose and Cellulose Industries" at Ahmedabad Textile Industry's Research Association, Ahmedabad 9, during January 29–30, 1962. Further information can be obtained from the Director, Ahmedabad Textile Industry's Research Association.

The name of one of the authors of the communication entitled "Mouse Lymphoma Cells with Different Radiosensitivities" in *Nature* of November 11, p. 572, was omitted; the communication was by Dr. P. Alexander and Z. B. Mikulski.

XUM

THE UNIVERSITY OF OXFORD EXPEDITION TO PERU, 1961

THE University of Oxford Expedition to Peru, 1961, has recently returned to the United Kingdom after completing approximately three months field work in the Central Andean Region south of Huancayo. The investigated area covered 600 sq. miles between latitudes 12° 25′ and 12° 50′ S., and longitude 75° 15′ and 75° 30′ W., at altitudes from 13,000 to 18,000 ft. o.d. Six scientists completed an integrated study which concentrated on the geological and botanical features of the country.

No previous work had been done on the geology of the area although the region was adjacent to country mapped by Dr. J. V. Harrison of the Department of Geology and Mineralogy, Oxford. R. C. Herrera constructed an initial geological map on the scale 1 : 50,000. This work was augmented by detailed stratigraphical sections of the Mesozoic and Tertiary, and by systematic collection of fossils, especially from the Cretaceous limestones. A small number of volcanic and acid intrusive specimens were collected for Prof. B. Giletti of Brown University, in conjunction with a programme of age determination of Andean rocks. The results of the investigation will be used by Dr. J. J. Wilson, of Lima, in his work on the Mesozoic history of the Andean Geosyncline.

The botanical work, carried out by J. R. Lloyd and J. K. Marshall, was divided into five parts. First, a collection was made of the flowering plants, ferns and bryophytes of the whole area. Concomitantly, a map and general survey of the plant formations in the region were completed. Thirdly, the quadrat method was used for a more intensive examination of samples from the formations of all districts. Completeness and precision were given to this phase by the detailed study of all the communities of a selected 10 sq. mile plot. Material comparable with the recently discovered fern-allay, *Stylites*, was found to be widespread in the *Distichia* bogs, and an autecological investigation was made of this and other genera. Finally, analysis of the changes of day and night temperature of four different flowering plant genera was carried out. The botanists were accompanied for a short time by Dr. O. Tovard, a Peruvian taxonomist, who aided in collecting and general survey.

S. R. S. Walker carried out work on the various soils, the majority of which were skeletal lithosols with a marked absence of horizons. His work was closely linked with the ecological programme of the botanists, and the samples will be analysed at the Department of Botany, Cambridge.

A 1 : 50,000 map of the north-eastern part of the area was compiled by the surveyor, M. R. Bewsher; this supplemented the smaller-scale published maps. Stadia methods were used to map at 1 : 5,000 a limited area of particular interest to the botanists. In addition, Bewsher recorded continuous meteorological observations at six stations.

M. J. Troughton undertook a geographical description of the study area, and in particular of the Ayhuicha Valley centred on the village of San José de Acobambilla. This is an isolated and particularly self-sufficient peasant community, and he was fortunate in being able to carry out the investigation during the 'faena' or collective community work period. At this time the men are recalled to the village from work among the herds on the puna grazing areas, thus presenting a good opportunity of observing the various activities of such a population.

Throughout the Expedition, great use was made of animal transport—mainly pack mules, which, in spite of their temperament, were ideally suited to the rough mountainous terrain. The mules, the extreme hospitality of the local Indians, and a working knowledge of Spanish were found to be great aids to exploration in the Andes of Peru. R. C. Herrera

ELECTRON MICROSCOPY IN THE UNITED STATES

THE nineteenth annual meeting of the Electron Microscope Society of America was held at the Pittsburgh Hilton Hotel, Pittsburgh, Pennsylvania, during August 23–26. The Local Arrangements Committee, headed by Dr. Robert V. Rice, is to be especially commended for the arrangement and success of this meeting, which brought together more than 706 active workers in electron microscopy from 24 American States, Canada, Australia, Great Britain, Belgium, Germany and Japan. It was the largest meeting in the history of the Society. There were ten general sessions of contributed papers, five in the biological fields, five in non-biological areas ; in addition, a special session on techniques and three symposia were features of the programme. Nineteen scientific exhibits were presented and twenty-one commercial exhibitors participated in the meeting. Some 180 authors contributed papers on the technique and applications of the electron microscope to work in biology, chemistry, medicine, cancer, metallurgy, electron diffraction and related fields.

A limited number of programme abstracts are still available from Dr. A. R. Taylor, Parke, Davis and Co., Detroit, Michigan. One hundred and three new members of the Society were elected, making the total membership 1,040.

In the symposium on "The Contribution of Electron Microscopy to Polymer Morphology", organized by Dr. R. G. Scott (Pioneering Research Division, DuPont Experimental Station, Wilmington 98, Delaware), seven experts in this field discussed X-ray diffraction applied to polymers, spherulites, electron diffraction applied to single crystals, morphology of polymers, fibre microscopy and electron diffraction applied to synthetic fibres. Arrangements for this symposium were planned over a period of two years and summarized the new phases of technology and applications of electron microscopy in the field of polymer science.

Dr. Rubin Borasky (Electron Microscope Laboratory, Graduate College, University of Illinois, Urbana, Illinois) arranged and directed the symposium on "Ultrastructure of Protein Fibers—Collagen, Muscle, and Keratin". The papers on physical, chemical and mechanical properties of protein fibres in muscle and keratin summarized the recent con-

tributions to the science of fibre structure, especially collagen.

The symposium on "The Fine Structure of Viruses" arranged by Dr A. F. Howatson (Medical Biophysics Department, University of Toronto, Toronto 5, Ontario) was especially noteworthy in that the presentation of papers on recent advances in fine structure, animal virus structure, electron microscopy of nucleic acids and proteins, structures of bacteriophages and their variations, and work on the fine structure of foot-and-mouth disease virus brought together in a very logical sequence the information on the recent improvements in the techniques of replication, shadow-casting, thin sectioning and "negative contrast" staining and their application and interrelationships in obtaining new information on the structure of viruses both *in situ* and isolated.

Fifth International Congress for Electron Microscopy

Arrangements for the Fifth International Congress for Electron Microscopy to be held in Philadelphia during August 29–September 5, 1962, were announced at the meeting.

The Electron Microscope Society of America will be the host for the International Federation of Electron Microscope Societies at this Congress to be held, coincidently, on the twentieth anniversary of the Electron Microscope Society of America. Efforts are being made to have the Congress include the most significant developments since the last Congress in Berlin in 1958. The general theme will emphasize new and unique contributions of electron microscopy toward the advancement of science. In addition to contributed papers, the programme is being organized to include special papers by invited speakers which will set the theme of each session. In the interest of the earliest possible dissemination of the new material to be presented at the Congress, the Committee has completed arrangements for each active member at the Congress to receive the bound copy of the scientific papers at the time of registration. This will contain an abbreviated paper, including some micrographs or illustrations, for each scientific contribution presented at the Congress.

Further information can be obtained from the Secretary, Fifth International Congress for Electron Microscopy, 7701 Burholme Avenue, Philadelphia 11, Pennsylvania.

A. R. Taylor

THERMIONIC CONVERSION OF HEAT TO ELECTRICITY

A SYMPOSIUM on the thermionic generation of electricity was held at Bankside House, London, on May 11. It was organized by the Central Electricity Generating Board and attended by workers in the field of thermionic generation from a number of public and private concerns in Britain.

An opening address by Dr. K. H. Spring, of the Research and Development Department of the Central Electricity Generating Board, was followed by a paper from Mr. R. V. Harrowell, also of the Board, and Dr. J. Myatt (Atomic Energy Research Establishment, Harwell) on the efficiency and power of a diode generator. This paper examined the simplified expression for efficiency:

$$\eta = \frac{\varphi_c - \varphi_A}{\varphi_c + P_L/I_A}$$

where φ_c and φ_A are cathode and anode work functions, P_L is the power loss and I_A is the anode current. When η is plotted against φ_c, the resulting humped curve is asymptotic to zero efficiency as φ_c is increased indefinitely; and for $\varphi_c = \varphi_A$, $\eta = 0$. It was pointed out that emphasis is seldom laid on the fact that this efficiency curve is valid only for a matched load at each value of φ_c: it is then true that $\eta = 0$ at $\varphi_c = \varphi_A$. But it is quite possible, under non-matched conditions, to have a high efficiency even when $\varphi_c = \varphi_A$, and this, or an intermediate state, may be desirable in certain circumstances. More important is the fact that, under matched conditions, maximum efficiency may sometimes be sacrificed for an increase in power; in this case φ_c will be less than its value at maximum efficiency.

A low anode work-function is important for the realization of high efficiency. The effective work-function of a metal anode in cæsium vapour depends on the cæsium coverage of the surface. A value below both the figure for clean metal and the bulk cæsium work-function can be achieved with a correct balance between the anode temperature and the saturated cæsium temperature: a value of 1·1 V. has been quoted.

A study of the work-functions of anodes opposite oxide cathodes has been made by Dr. B. J. Hopkins (University of Southampton). The work-function of a clean nickel anode (~ 4·7 V.) fell to less than 2·5 V. when the cathode was in the most active condition. Dr. Hopkins considers that barium oxide evaporates from the cathode in the molecular form and is deposited on the anode. Three methods of measurement were used: two depend on the determination of the contact potential difference from retarding field curves; the other uses the contact potential difference obtained by the Kelvin method. If the contact potential difference between the cathode and a known reference surface is also measured, then the anode work-function can be found by eliminating the cathode work-function from the two expressions; alternatively, the cathode work-function can be measured from a Richardson plot, although this would give a less trustworthy result.

Mr. D. C. Gore (Central Electricity Generating Board) read a paper entitled "The Thermionic Diode in Large-Scale Power Production from Fossil Fuels". This dealt with the diode as a thermodynamic topping device in a conventional boiler, using the upper part of the available temperature-range, which is not usable in a steam cycle. The mechanical arrangement would be for the diodes, in cylindrical form, to envelop the boiler tubes; the outer, cylindrical cathodes would be heated by the furnace gases. If a diode efficiency of 20 per cent could be attained and the efficiency of the conventional plant were 35 per cent, the total plant efficiency would be raised to 45 per cent by the introduction of diode 'toppers' passing three-quarters of the available heat. Any saving in fuel costs would probably be considerably offset by the relatively short life of the diodes, but Mr. Gore believes that if the very difficult problems of materials can be solved there are reasonable prospects for reducing generating costs. Some of the problems

XUM

of materials and techniques were then reviewed by Mr. E. Cohen (Central Electricity Generating Board).

Mr. D. A. Watt (Atomic Energy Research Establishment, Harwell) and Mr. J. A. McWilliams (Boucher and Co., Kidderminster) described two engineering design studies of experimental in-pile thermionic generators: one has a planar geometry and uses ceramic–metal seals; the other, with a cylindrical geometry, has been simplified by omitting the ceramic–metal seals and using a continuous stainless steel envelope, which also forms the anode load. In the planar device the cathode is a coating of natural or depleted uranium carbide–zirconium carbide (25 : 75) on a tantalum 'top-hat', the inside of which contains uranium carbide–zirconium carbide enriched with uranium-235 as the fuel. To overcome the serious compatibility problem of fuel material in contact with tantalum at 2,000° C., the cylindrical version uses a rod of uranium carbide–zirconium carbide made of depleted or natural material at the end in contact with the tantalum cathode support and enriched material as the fuel at the free end. An output of about 300 W. was expected from an experimental model of the cylindrical diode being made.

The use of ion injection for space charge neutralization was discussed in a paper by Mr. G. G. Isaacs (G.E.C. Hirst Research Centre). In this type of device, ions are generated by an auxiliary discharge and, therefore, inert gases may be used instead of the chemically active alkali metal vapours. The experiments reported indicated a high degree of neutralization of space charge. A net output of about 200 mW. was obtained at an efficiency of about 1 per cent; however, no attempt had been made to optimize the design. As well as freedom of choice of filling gas, a further advantage of this kind of device is its potentiality for generating alternating current.

Mr. J. A. Thorp (A.E.I., Rugby) spoke on "Auxiliary Ion Sources in Cæsium Diodes". One method of neutralization of space charge is to produce positive ions from cæsium atoms in contact with a hot surface the work-function of which exceeds the ionization potential of cæsium. Mr. Thorp dealt with an investigation using matrix-type cathodes of low average work-function but with some high work-function spots at which ionization occurs. It is possible, however, for some ions to be trapped in a potential well at the mixed work-function surface and thereby be rendered ineffective. In the work described, a hot tungsten filament between anode and cathode has been used to ionize the cæsium. When the filament was biased one or two volts positive relative to the cathode, the anode current was several times greater than when the ionizer was unbiased. This indicated that the potential well at the filament was removed and positive ions were released.

In an analysis of the processes of ionization and de-ionization in a diode, Dr. L. G. Sanders (Atomic Energy Research Establishment, Harwell) considered the comparative effects of ionization by electron collisions and photons, and the so-called resonance ionization (also used by Mr. Thorp). It appears that the first two types of ionization are small compared with resonance ionization at the cathode, provided, in the case of electron collisions, that the electrons are at cathode temperature. However, if the electrons are at a temperature two or three times greater than the cathode temperature of about 2,000° C., electron collision ionization can make an appreciable contribution. The electron temperature may well be of this order due to barrier and Thomson heating. A simple approach, based on the current balance at the electrodes, has shown that the highest vapour pressure necessary is that which just neutralizes the negative space charge. If the pressure has to be higher than this amount in order to lower the cathode work-function by partial cæsium coverage, then the electrode spacing should be decreased to limit the plasma resistance.

In a review of progress in the United States, Dr. B. C. Lindley (C. A. Parsons and Co., Ltd.) reported that the trend there is towards the use of refractory metal cathodes and high cæsium pressures: the former enables high cathode temperatures to be used, while the high vapour pressure ($\sim$ 10 mm. mercury) ensures, even at 2,000° C., a reduction in work-function due to the presence of a monolayer of cæsium; electrode spacings of the order of the electron mean free path minimize heat conduction through the plasma. Most of the American groups visited were working on the close-spaced vacuum converter as well as the cæsium version.

"The Crossed Field Thermionic Converter" was the title of the final paper, which was read by Dr. G. D. Sims (University College, London). In this device, space charge is overcome by crossed electric and magnetic fields. Dr. Sims described experiments that have established beam interaction with the accelerator electrode in the initial apparatus used. He believes there is little doubt that the accelerator current, which persists even with extremely high magnetic fields, arises as a result of instability in the electron cloud; this gives rise to either amplification of noise or oscillation—resulting in radio-frequency fields which cause electrons to lose sufficient energy to be drawn to the accelerator. This represents wasted power. Further experiments are being planned with the view of the suppression of accelerator current.

R. V. Harrowell.

RESEARCH IN THE LEATHER INDUSTRY

THE seventh biennial conference of the International Union of Leather Chemists Societies was held in Washington, D.C., during August 20–25.

The International Union was founded in Europe after the Second World War as a confederation of national member societies concerned with the leather industry, and research and technology related to it. From an early stage in its development, it had as one of its members the American Leather Chemists' Association, and in the twelve years since its foundation it has come to include among its twenty-five member societies a steadily increasing number from Asia, Africa and South America, the expanding leather industries of which are based on locally available supplies of hides, skins and tanning materials. The decision to hold this year's biennial conference outside Europe for the first time, and to combine it with the annual meetings of the American Leather Chemists' Association, is evidence of the growing collaboration between American and European leather chemists and their colleagues in other continents. Thanks to its efficient organization by the host society,

an enjoyable, lively and instructive conference resulted.

As a craft industry, organized in relatively small units, the leather industry has in the past paid too little attention to the more fundamental types of research, and is to-day still handicapped in competition with the producers of synthetic and substitute materials by the allocation of insufficient funds to research and development. Not only the scale of research effort needs to be increased, however, but also the communication of the results in a form in which they are assimilable and can be applied in tanneries. These points were emphasized by M. Riviere (Strasbourg) in the John Arthur Wilson Memorial Lecture with which the conference began, and also by speakers in the ensuing discussions, public and private. It was evident that there was a deeply felt need for closer collaboration between research workers and the industry, and that while national and international conferences such as the present one help to disseminate scientific information among the research workers, they do not necessarily ensure that this information is put to the best practical use in the industry itself; to achieve this, a programme of education is needed to acquaint the tanner with scientific discoveries which he can use, but also to acquaint the scientist with the needs and difficulties of the industry.

Of the sessions which were devoted to scientific papers, the outstanding ones were those devoted to work on the chemistry and structure of collagen. The president of the Union, W. Grassmann (Max-Planck Institut, Munich), reviewed present knowledge of this subject as a preliminary to a discussion of the results recently obtained by his group. The light and dark bands revealed by electron microscope photographs of collagen fibrils have different amino-acid compositions. By the investigation of peptides isolated from heat-denatured collagen by degradation with trypsin, it has been shown that some regions of the peptide chains contain more of the polar amino-acids than occur elsewhere, while in other regions the amino-acids are mainly glycine, proline and hydroxy-proline, with some alanine and serine. In the non-polar regions, the sequence is usually glycine–proline–X, where X is either glycine, serine or alanine. In the polar regions, which give the dark bands, the sequence is less regular, and glycine does not occur at every third position. Carefully controlled denaturation of tropocollagen (soluble collagen obtained by leaching young skins in mildly acidic conditions) results in the production from collagen (mol. wt. 360,000) of α-collagen of mol. wt. 110,000 and β-collagen of mol. wt. 215,000 in equal amounts, and the denaturation is thought to cause multiple breaks in the rod-like tropocollagen, following the uncoiling of the collagen helices. According to F. O. Schmitt *et al.*, the long spacing segments are built up of parallel bundles of tropocollagen molecules, whereas in the fibrils of native collagen, neighbouring molecules are displaced relative to one another by one-quarter of their length, and this view has been confirmed by staining techniques followed by photometric measurements along the lengths of the segments. The collagen molecules are bound together laterally by covalent cross-links, which are the cause of the limited swelling and the insolubility of collagen in water. As was shown in a paper by H. Hörmann (Max-Planck Institut), the covalent bonds consist of hexoses the two hydroxyl groups of which form ester links between different peptide chains in collagen. Grassmann indicated that tannage consists generally of an increase of cross-linking of molecular chains within dark band regions of fibrils, although some tanning materials also form compact deposits surrounding the fibrils, and without any true tanning action.

K. Kühn (Heidelberg) described experiments on the end-to-end linkage of tropocollagen molecules, involving their treatment with trypsin and pepsin to remove the peptide appendages which are thought to protrude from the ends of these molecules. The results were held to indicate that the end-to-end polymerization of tropocollagen molecules was mainly due to electrostatic forces, but this view was questioned by Schmitt in the discussion following the paper. The production of gelatin fractions from skin collagens, and their re-constitution as fibres, was described in a paper by A. Veis and J. Anesey (Northwestern University, Chicago) with J. Cohen (Armour Leather Co., Chicago), while A. G. Ward, N. T. Crosby, D. G. Higgs, R. Reed and G. Stainsby (University of Leeds) described experiments the aim of which was to break the insolubilizing cross-links of mature bovine collagen after alkaline pretreatment. The rod-like particles of collagen may be dissolved to yield products similar in many respects to those from young skin. Some beautiful electron microscope photographs were shown by L. P. Witnauer and L. D. Kahn (Eastern Regional Laboratory, U.S. Department of Agriculture, Philadelphia); collagen fibrils precipitated by inorganic ions from solution had various solubility characteristics, as well as a variety of forms.

Of the other twenty-five papers presented at the conference, the majority were concerned with leather technology rather than with questions of general scientific interest, and reference can be made to only a few of them. H. G. Turley (Rohm and Haas Co., Philadelphia) compared and contrasted American and European practice in chrome tanning, and indicated that the different methods used are determined by economic factors as much as by different objectives. P. J. van Vlimmeren (T.N.O., Waalwijk, Holland) described work on masked chrome tannages, stressing the importance of the stage at which the masking agent (formate, phthalate, acetate or oxalate) is added to the tanning liquor. Because of the different rates at which chrome complexes are formed, the most appropriate stage differs for various masking agents and, when chrome sulphate alone is used, it may be best to add the material to pickled pelt as a dry powder produced by the slow evaporation of chrome sulphate solution; this gives strongly masked anionic complexes which break down only slowly in cold solutions. E. Heidemann, P. Führen and H. Kröll (Technische Hochschule, Darmstadt) discussed the tannages of synthetic polypeptides (used as model substances) with chrome. They have produced them as gelatinous materials or as voluminous flakes in which all the carboxyl groups are probably accessible to chrome complexes, and their results suggest that the majority of the cross-linkages between carboxyl groups involve complexes containing a pair of chromium atoms.

In a session devoted mainly to the physical testing of leather, E. Baumann (Bally Shoe Factories, Schoenenwerd, Switzerland) stressed the need for simple and rapid test methods, which are essential in testing a biological material such as leather for factory control. The abrasion testing of sole leather was discussed by R. G. Mitton, M. Millar and F. R.

XUM

Morgan (British Leather Manufacturers' Research Association), who showed that, despite the obvious complexity of the wearing process of shoe soles, there is a good correlation between the results obtained in abrasion tests and durability measured in wear trials of shoes. In favourable cases, the closeness of the correlation seems to be limited by the unavoidable errors associated with the wear trials (and amounting to a few per cent) rather than to any other cause.

R. G. Mitton

INTERNATIONAL UNION OF FOREST RESEARCH ORGANIZATIONS

THE thirteenth Congress of the International Union of Forest Research Organizations was held in Vienna during September 11–16 under the presidency of Mr. James Macdonald, director of research and education in the Forestry Commission, London. Vienna was the birth-place of the Union, and the small body of specialists, members of Austrian, German and Swiss forest research institutes, who first met (at Mariabrunn) in 1893, would surely have been gratified at the ramifying growth and vigour of their brain-child. More than 400 delegates, an all-time record, attended the Congress from about three dozen countries, with particularly strong contingents from Finland, Germany, Japan, Scandinavia, the United States and the U.S.S.R.

All working sessions were held at the Wilhelm Exner Haus, Hochschule für Bodenkultur, and the opening and closing ceremonies at the more central Haus der Industrie. The Congress was formally opened by the Federal Minister of Agriculture and Forestry, who reviewed the great changes—territorial losses, damage, over-exploitation and neglect—wrought by two World Wars in the forest economy of Austria and emphasized the growing importance of the forests, both State and private, not only as suppliers of timber, internally and abroad, but also as stabilizers of mountain slopes and guarantors of the water supply. The Marshall Plan had given a great impetus to rehabilitation, and now an extensive programme of torrent- and avalanche-control, embracing both engineering works and reforestation, was under way, backed by a carefully conceived plan of research.

The main work of the Congress was, as usual, carried out in the sections, of which (inclusive of bibliography) there were ten, which constitute the living heart of the Union. The section leaders, who continue in office between Congresses, are responsible for keeping in touch with the research workers in their respective fields of action, for distributing information of interest to specialist working groups and other members of their section, for initiating action as necessary, and preparing for and conducting their sectional meetings at Congresses.

Section 11 (Forestry Influences), under Dr. H. Storey of Washington, dealt with three main topics: management of forested watersheds for water supply (where stress was laid on the need for new measurement techniques and instrumentation), the role of forests and engineering structures in the control of floods and erosion (emphasizing the supplementary functions of biological and engineering methods) and shelterbelts, and ended with appointing working groups to initiate and co-ordinate work in each of these fields. Section 21 (Site Factors), under Prof. de Philippis of Florence, had forest typology, the influence of forest stands on the soil, and biological factors of forest regeneration as its main topics, and set up a joint working group with Section 25 in order to study what are the best criteria for assessing the quality of sites and the relationships between environmental factors and the potential productivity of sites. In Section 22 (Forest Plants) under Dr. C. Syrach Larsen of Hørsholm, the chief topics discussed were seed production problems in forest trees and the growing of pines in the tropics and subtropics; a working group was formed on provenance research. This Section has hitherto concentrated on tree breeding, but is becoming increasingly interested in physiology. Dr. Leibundgut of Zurich presided over Section 23 (Silviculture), in which a great variety of subjects was discussed, notably: silvicultural problems raised by the general unsaleability of small-dimensional timber—a topic of world-wide interest—for which a new working group was set up; silvicultural problems of tropical forests; soil amelioration and weed control in nurseries; and root formation and competition. This Section is also busy, in collaboration with the Bibliographical Section, on terminology in its field. Section 24 (Forest Protection), under Prof. A. Biraghi of Florence, dealt mainly with insect population dynamics—especially the influence of manuring of forest stands on the development of populations of noxious insects, about which there is some controversy; mycorrhiza research; international co-operation in forest disease research and in quarantine measures; and problems of smoke and industrial fumes damage to forest growth. It also created a fifth working group to study the important question of fire control.

Section 25 (Growth and Yield), under Prof. S. Firat of Istanbul, discussed mainly: the use of climate site indices—concluding that these cannot by themselves afford an accurate guide to the quality class of individual forest sites and setting up a joint working group with Section 21 (see above); the use of electronic digital computers in forest research—recommending *inter alia* the compilation of a register of existing computer programmes of interest to foresters and an index of organizations and individuals that are actively using computers; increment determination in uneven-aged tropical forests (a matter of great present-day interest) and increment determination by borings—in both of which, it was agreed, investigations were not sufficiently advanced to permit anything but tentative conclusions. This Section has a permanent working group that advises the Union on all matters concerned with aerial photography, photo interpretation and photogrammetry, and maintains effective links with Commission 7 of the International Society of Photogrammetry; the report of this group was approved and its membership is being widened. The Section also recommended the formation by mathematical specialists of advisory groups for helping forest statisticians, both by suggesting improvements in current university curricula on statistical methods and by organizing the collection and distribution of case studies and other information of value for the statistical planning of forest research. Prof. J. Speer of Munich led Section 31 (Forest Economics), which formulated and recommended a special accounting system for forest com-

mercial enterprises and a corpus of economic terms that could be used by all countries. It also discussed the difficult topic of the measurement of forest work productivity with Section 32 (Operational Efficiency) the other main topics of which, under the chairmanship of Prof. V. Sundberg of Stockholm, were the effects of noise and vibration on the forest worker and the establishment of university courses in operational efficiency. Section 41 (Mechanical Conversion), led by Prof. J. Campredon of Paris, discussed many aspects of this subject and decided to enlarge its field of study so as to embrace all forest products (this to be the new name of the Section), with three initial working groups for wood quality, behaviour of wood exposed to fire, and sawing and machining.

Finally, on the initiative of Austria and Germany, the Union decided to create a new section, Section 11, on the History of Forestry, with Prof. von Mantel (West Germany) as leader.

The Bibliographical Section, which is actually a joint committee of the Food and Agriculture Organization and Union representatives, met as usual several days before the Congress, under the chairmanship of Prof. E. Saari of Helsinki. It was concerned mainly with the prosecution of two long-term projects—further accurate translations of the classification now widely accepted in the world of forestry science (*The Oxford System of Decimal Classification for Forestry*, of which English, French, German and Spanish authorized editions have already appeared) and speeding up the production on cards of a basic forest terminology in the English language, as an essential preliminary to the multilingual terminology project, sponsored jointly by the Food and Agriculture Organization and the Union.

Prof. J. Speer (West Germany) was elected to be president in succession to Mr. Macdonald, whose term of office expires at the end of the year. Dr. V. L. Harper (United States) was elected vice-president. Elections to the Permanent Committee (previous members in italics) were: Prof. N. Anutschin (U.S.S.R.), *Prof. T. Bunusevac* (Yugoslavia), Prof. J. Campredon (France), Dr. E. Holmsgaard (Denmark), *Prof. A. Horky* (Austria), *Prof. M. Kreutzinger* (Poland), Prof. A. de Phillipis (Italy), Dr. D. R. Redmond (Canada), Dr. H. Shirley (United States), and Dr. Saito (Japan), leaving two places to be filled by representatives from India and Latin America, not yet designated.

The International Council met on the concluding day, and agreed to the revised statutes which take into account recent decisions of and developments in the Union, the major changes being to strengthen the position and powers of the enlarged Permanent Committee which is its executive body between Congresses. A new class of member—associate member—has also been created and the organizing of the secretariat, following its recent relinquishment by the Food and Agriculture Organization, has been entrusted to the president himself.

The Union accepted an invitation from Germany to hold the next Congress there. After the Congress at Vienna, about 130 of the delegates took part in strenuous study-tours that lasted for 11 days of magnificent weather and covered the whole country. One tour concentrated on silviculture and management, another on the many aspects of forest protection, and the third on mechanical developments in soil-cultivation and trenching, tree planting and tending, timber extraction and milling.

F. C. Ford-Robertson

NUTRITION AND POULTRY

THE Scottish Group of the Nutrition Society held its sixty-second meeting on September 30 in the College of Agriculture, University of Edinburgh. It took the form of a symposium entitled "Nutrition and Poultry". As the chairman, Prof. S. J. Watson (University of Edinburgh), pointed out in his opening remarks, since the subject was "Nutrition *and* Poultry" it was not confined to problems of poultry feeding but instead embraced broader aspects of nutrition in which work with poultry has contributed to knowledge.

Dr. W. P. Blount (British Oil and Cake Mills, Ltd., London) opened the proceedings with a very thorough exposition of the use of birds as experimental animals. He said that apart from some cage birds which have special uses, poultry were the most commonly chosen birds for laboratory work, and he emphasized the advantages of their cheapness, availability and ease of management. Illustrating his talk with a series of informative slides, he described different forms of housing, from brooders for small groups of chicks to intensive houses for many thousands of adult birds. He discussed optimal conditions of light, temperature and humidity, systems of feeding and ingredients of poultry diets. He concluded with a survey of the common diseases of poultry, and indicated that many of them could be controlled by good management and the judicious use of drugs such as antibiotics and coccidiostats.

In the following paper Dr. M. E. Coates (National Institute for Research in Dairying, Shinfield) dealt with problems of fundamental nutrition for which poultry have proved peculiarly suitable subjects, particularly in vitamin research. The examples quoted ranged from Eijkman's classic observation of polyneuritis in hens fed on polished rice, which led to the discovery of vitamin B_1, to modern work on the isolation and function of vitamin B_{12}, a field in which work with poultry has played a major part. The developing chick embryo was cited as a very suitable organism for investigating the biochemical role of nutrients, as evidenced by experiments on the metabolism of vitamin B_{12} and studies in tissue culture of the function of vitamin A. She described how chicks can be relatively easily maintained in a germ-free state and are thus becoming increasingly important in investigations of the relation of the alimentary microflora to nutrition.

Prof. S. Lepkovsky (University of California), who has used birds to study the nervous control of food intake, gave a fascinating dissertation on hunger, appetite and satiety. He described the influence of the central nervous system through hunger- and satiety-centres in the hypothalamus. He illustrated the psychological effects of these states amusingly and accurately in a series of lantern slides of "Hubert", an unfortunate character from a comic cartoon who was obliged to subsist on a low-calorie diet.

It has become common practice to include in diets for livestock non-nutritional additives that increase growth-rate and level of production. In reviewing additives for poultry diets Dr. J. H. Taylor,

XUM

(Cyanamid Products, Ltd., London) classified them into those that alter the microbial flora to the benefit of the host animal and those, largely having hormonal activity, that produce increases in growth or productivity by modification of metabolic processes. The former class of compounds was of greater practical importance, and had the largest effect when the level of performance was poor. Detectable quantities of some antibiotics given in the diet appeared in the serum. If there was reason to suppose that the beneficial effect of the antibiotic was systemic it was desirable that blood-levels should be increased. If, however, the response to an antibiotic was solely due to modification of the gut flora, such enhancement was unnecessary. The hormonal food additives such as œstrogens and thyroid-active agents could be of use in increasing fat deposition and improving carcass quality, although there was some danger of residues in the tissues if œstrogens were given in the food.

The final paper in the symposium was an entertaining account by Prof. M. F. M. Meiklejohn (University of Glasgow), speaking in his capacity as editor of *Scottish Birds*, of wild birds that have been used as food for man. He began by reading from the *Book of Leviticus* the catalogue of birds that were prohibited as food by Mosaic law, and showed how nearly it corresponded to the list of birds customarily considered as unfit for consumption. The choice of wild birds for human food depended on their palatability and the ease with which they could be captured. In general, sea birds were less palatable than land birds, although there were instances of inhabitants of remote islands subsisting on some of the colonial nesting species. Of the land birds, those that lived in flocks and hence were more easily caught in quantity were most frequently a source of human food. Many of the traps and nets used to-day by ornithologists derived from the catching devices evolved long ago for the capture of wild birds for food. Prof. Meiklejohn posed a number of questions, which the audience attempted to answer, about the relation between the food of wild birds and their suitability for human consumption.

The lively discussion that followed each of the papers fully justified the choice of subject for this symposium. On this occasion at least, the more homely aspects of the domestic bird were overshadowed by its role in the field of nutritional science.

M. E. Coates

THE EUROPEAN NUCLEAR ENERGY AGENCY

THE third report of the European Nuclear Energy Agency*, presented to the Consultative Assembly of the Council of Europe by Prof. S. Balke on September 26, records substantial progress in the first joint undertakings. Work on the Eurochemic site was inaugurated in July 1960 and the detailed preproject for the plant was approved in June 1961. The Halden heavy-water reactor reached boiling in October 1960 with the first fuel charge of natural uranium, and installation of a second fuel charge of enriched uranium will permit a power of 20 MW. to be reached before the end of the year: joint operation of the reactor has been extended to the end of 1962. Construction of the *Dragon* high-temperature gas-cooled reactor began in April 1960: civil engineering work on the site is well advanced and supporting research and development are progressing in parallel.

A group was set up in November 1960 to study the nuclear propulsion of ships, and a meeting of experts has been convened to determine the areas of nuclear research where particular need for international co-operation exists and the objectives and means of collaboration.

The European–American Nuclear Data Committee, created in June 1959, reviews the measurement programmes of member countries, inventories the equipment available, and has compiled a list of requests for new measurements so as to facilitate collection of the necessary data. It is also assisting countries which have only recently begun to work in this field, and the Agency, in July 1961, decided to establish a European system of supervision and emergency warning which involves the immediate notification of any abnormal increase in environmental radioactivity recorded in a member country. A study-group on food irradiation was set up in January 1960 to examine the economic and technical possibilities of the application of food irradiation so as to harmonize national programmes and propose joint projects where appropriate. International seminars for university teachers and technical executives were again organized in 1960 and 1961, and a specialized symposium on criticality control in nuclear industry at Karlsruhe was attended by one hundred specialists.

The Agency has also undertaken the examination of regulatory, administrative and legislative provisions introduced by European countries to conform with the radiation protection norms adopted in 1959, and these norms are being revised, in liaison with Euratom and the International Atomic Energy Agency, to take account of the latest recommendations of the International Commission on Radiological Protection. A Convention on Third Party Liability in the Field of Nuclear Energy, dated July 29, 1960, has been signed by sixteen members of the Organization for European Economic Co-operation. The Agency also participates in the investigation of problems posed by the insurance of nuclear risks, and particularly the application of tax regulations governing technical reserves and insurance premiums to these risks. A first set of regulations applicable to research or experimental reactors has been elaborated by the Control Bureau responsible, under the Agency's Steering Committee, for carrying out the Security Control Convention. In addition, the Agency centralizes economic data bearing on the development of the nuclear industry, and the result of an investigation, published in 1960, predicted that nuclear power stations will provide less than 3 per cent of the capacity of the European electricity production in the coming five years and less than 10 per cent in fifteen years.

The practical value of international co-operation in this field is fully established by the year's work, and the experience gained in co-ordinating scientific work

* Organization for European Economic Co-operation—European Nuclear Energy Agency. Third Report on the Activities of the Agency. Pp. 253. (Paris: Organization for European Economic Co-operation–European Nuclear Energy Agency, 1961.)

shows that in this way the less advanced countries gain indispensable experience that they could not acquire by themselves, while the other countries also benefit. This effort should be continued on as wide a geographical basis as possible, for the value of the results is increased by the scope and variety of the means used and relative costs are reduced. The report emphasizes that this experience in nuclear research is valid in other scientific fields and that European scientific progress calls for a concentration of human, financial and industrial resources only attainable by international co-operation. Annexes to the report include a report on the possibilities of joint European action in nuclear propulsion of ships and L. Kowarski's report on new trends in atomic research and their international significance.

UNIVERSITY FINANCE IN BRITAIN: 1959–60

THE annual returns from universities and colleges in Great Britain* in receipt of Treasury grants for the academic year 1959–60, now covering twenty-one universities and three colleges, is prefaced by a somewhat longer general survey than last year, in which university expenditure on research and academic salaries is considered more particularly.

The University Grants Committee has already made a recommendation to the Chancellor of the Exchequer regarding the location of a fourth new university, in addition to those recommended in July 1960, to be located at Colchester, Canterbury and Coventry, but this location is not disclosed. The Committee considers that the establishment of any new universities beyond these four should wait on the report of the Committee on Higher Education, and meanwhile academic planning boards are being appointed for the Universities of Essex, Kent and Warwickshire. It is expected that the expansion proposed by existing Scottish universities will provide the additional places needed in Scotland by the early 'seventies. Apart from this, the University Grants Committee emphasizes the importance of the enthusiasm and interest of a sponsoring area in determining their decisions on the location of new universities, as well as the presence of industries and some local financial support, the supply of lodgings and the attractiveness of the location to academic staff.

Following Sir Arthur Rucker's review of the application by the universities of the procedures recommended by the Gater Committee, a Building Sub-Committee has been established under the chairmanship of Sir Keith Murray to advise the University Grants Committee on its procedures for assessing non-recurrent grants for building works. A sub-committee has also been set up to assess, and advise the Committee on, the proposals for development in Oriental, African, Slavonic and East European studies which come forward from the universities, and a committee has been appointed with Sir Edward Hale as chairman, to make a comparative study of the methods of teaching undergraduates now being used in the universities and colleges of Great Britain in the fields of arts and pure and applied science. The report was obviously written before the Chancellor of the Exchequer made his statement in July, and it remains to be seen whether and how far the provisional building programme which the Committee has agreed with each of the universities, in the light of the Chancellor's statement on January 25, 1961, for each of the four years 1962–65, will be affected. To meet the expansion to 170,000 students by the early 'seventies and make good the backlog of past years, a building programme of £175 million at 1960 costs is required. The Committee has no apprehensions on the quality of students expected in the next ten years; it stresses again the importance of halls of residence; it welcomes the attention which the universities are giving to their existing degree structure, including the postgraduate structure, and generally it is impressed and encouraged by the attitude of the universities to the projected expansion.

The report records a solution to the question of assistance for St. David's College, Lampeter, by association with the University College of South Wales and Monmouthshire, which will assume sponsorship for St. David's for a trial period of ten years, during which, from August 1, 1961, the University Grants Committee will consider applications for recurrent and non-recurrent grants.

The number of full-time students in British universities again increased, to 104,009, compared with 100,204 in 1958–59, and statistics collected in October 1960 show a university population of more than 106,000. There were 6,443 full-time and 2,400 part-time students from overseas within the British Commonwealth, and 4,208 full-time and 2,068 part-time students from foreign countries. Of full-time men students 36·8 per cent were in arts, 24·7 per cent in pure science, 19·7 per cent in technology, and 11·9 per cent in medicine; these figures compare with 36·8, 24·2, 19·5 and 12·5, respectively, in 1958–59. For full-time women students the corresponding figures for 1959–60 are 62·0, 21·1, 1·3 and 11·8, respectively, and for 1958–59, 62·6, 20·7, 1·0 and 11·9. Full-time advanced students of pure science numbered 4,512 (34·5 per cent); of technology, 2,442 (18·7 per cent); and of medicine, 1,052 (8·1 per cent); 3,585 students were taking postgraduate courses in teacher training.

Of the full-time students, 83,083 were reading for a first degree, 3,605 for a first diploma, and 16,649 were engaged in research or other advanced work; the corresponding figures for 1958–59 were 80,805, 3,928 and 14,839, respectively. Of the new full-time students, 25·2 per cent were in pure science, 16·0 per cent in technology and 9·0 per cent in medicine; in 1958–59 the corresponding figures were 24·1, 16·2 and 8·9 per cent, respectively. Of all full-time students, 76·0 per cent were men and 24·0 per cent women, a proportion practically unchanged from 1938–39, when the corresponding percentages were 76·7 and 23·3; 17·2 per cent were at the Universities of Oxford and Cambridge, 20·9 per cent at the University of London, 39·1 per cent at other universities and university colleges in England, 5·9 per cent in Wales and 16·9 per cent at Scottish universities. The proportion of full-time students residing in colleges or halls of residence was 27·0 per cent,

* University Grants Committee. Returns from Universities and Colleges in receipt of Treasury Grant, Academic Year 1959–1960. Pp. 76. (Cmnd. 1489.) (London: H.M.S.O., 1961.) 4s. 6d. net.

XUM

compared with 26·6 per cent in 1958–59; 50·3 per cent were in lodgings and 22·7 per cent at home, but these proportions vary widely between men and women and between institutions.

The proportion of assisted students was 82·4 per cent, compared with 80·5 per cent in 1958–59 and 71·9 per cent in 1953–54, ranging from 93·6 per cent in Wales, 89·0 per cent in English universities, excluding those at Oxford, Cambridge and London, to 73·4 per cent for Scotland. Full-time teaching and research staff increased to 11,474 (10,823 in 1958–59) and 25·6 per cent are now in pure science, 15·0 per cent in technology and 17·3 per cent in medicine.

Of the recurrent income of £59,800,092 (£52,273,306 in 1958–59), £43,170,107 was from Parliamentary grants (72·2 per cent), £5,990,466 from fees (10 per cent), £1,731,106 (2·9 per cent) from endowments, £1,533,673 (2·6 per cent) from local authority grants, £451,328 from donations and subscriptions, and £4,730,590 from payments for research. Non-recurrent Treasury grants amounted to £19,852,747, an increase of £3,298,264 on the previous year, and of this, £13,839,308 was spent on building work, £1,914,375 on professional fees, £2,966,333 on furniture and equipment and £1,132,731 on site and property purchases. Of the recurrent expenditure of £58,505,072—an increase of £3,978,797 on that for the period 1958–59—6·8 per cent was spent on administration, 44·0 per cent on salaries and superannuation of teaching staff, 11·1 per cent on departmental wages, 13·9 per cent on departmental and laboratory maintenance, 3·6 per cent on repairs and maintenance of buildings and 9·7 per cent on rates, insurance, heat, light, etc. Expenditure on libraries, at £2,350,294, remained at 4·0 per cent of the total.

HEALTH SERVICES IN SMALL FACTORIES

A CONFERENCE on "Health Services in Small Factories" was convened jointly by the World Health Organization Regional Office for Europe and the International Labour Organization at Dun Laoghaire, Dublin, Ireland, during May 2–16 (*WHO Chronicle*, 15, No. 9; 1961) The forty participants in the conference included medical officers, officials from Ministries of Labour, and representatives of employers' and workers' organizations from more than twenty countries.

Between 80 and 90 per cent of factories in most European countries employ less than 100 workers. Of the 220,000 factories in the United Kingdom, 150,000 employ 10 or fewer workers; more than a million people work in factories employing 25 or less, the number of such factories having nearly doubled in the past twenty-five years. In Italy, 95 per cent of industrial plants have less than 100 workers, in Switzerland 90 per cent, in Belgium 96 per cent, in Denmark 99 per cent, and in Sweden 92 per cent.

While the problem of providing health services in large factories in Europe is well on the way to solution even though different methods are used in different countries, the smaller factories in most countries of Europe do not have health services on or near their premises. The risks to which their workers are exposed are certainly not less than in the larger factories and in many cases are greater, since the working environment in terms of atmosphere, ventilation and layout is considerably poorer.

Instruction in special hazards and supervision and training in handling dangerous materials, such as toxic chemicals or radioactive isotopes, are less efficient as a rule than in large factories. While periodic medical examinations are compulsory for workers in certain dangerous trades, there are usually no medical facilities within the factory beyond a first-aid box—the foreman is often in charge of this, but he is usually untrained in health matters.

There was general agreement among the participants in the conference that the provision of health services to these small undertakings is a real and urgent need, but there was less agreement about how to provide them. Among the difficulties are the diversity and dispersion of factories, which may be scattered over considerable distances. It is feasible for a doctor to provide health care for 3,000 workers in one factory, but not for thirty factories each with 100 workers. The cost of an autonomous health service is prohibitive for the small employer, and health hazards are often not understood or appreciated by employers and employees.

The conference discussed schemes now being tried out in various parts of Europe to provide health services for small factories. Four main tendencies are discernible.

In Czechoslovakia and in eastern Europe as a whole, these occupational health services are provided as part of a general programme of health protection covering both curative and preventive medicine and organized by the State.

In France, the Ministry of Labour is responsible for implementing general regulations on hygiene and security. Employers are obliged to set up health services which, for the majority of small factories, take the form of inter-factory services. The responsibilities of the physicians in these services are, in principle, the same as those of all industrial medical officers, that is, they are entrusted with preventive medicine, treatment being provided only in emergencies and for accidents.

In Italy, health services in small factories are mainly the concern of the National Institute for the Prevention of Accidents, which has some thirty branches scattered throughout the country.

In the Netherlands, the United Kingdom and Scandinavia, inter-factory occupational health services are provided on a voluntary basis, generally by means of a non-profit-making body on which doctors, employers and workers are represented. Organizations of this type show a tendency to grow in size and popularity as new factories join the service, and to extend to other areas. Their role is mainly preventive; but in some cases, they provide treatment also.

In a separate statement, the employers' representatives attending the Conference declared that, while agreeing that medical services in small factories should essentially be preventive, they nevertheless felt that these services should be encouraged to provide other treatment as well as emergency treatment. The advantages to be gained are a more efficient use of man-power, an increase in production

(since workers would not lose so much time visiting hospitals for treatment) and improved morale. They stressed their opinion that such services should be organized on a voluntary basis.

The representatives of the workers' organizations welcomed the important developments now taking place in the field of health supervision for their members, though many of them considered that progress in this field was still too slow. It was their opinion that, by accepting the goal of occupational health services for all workers, the International Labour Organisation has opened an important chapter in the history of occupational health.

UNIVERSITY OF SUSSEX

THE first prospectus* of the University of Sussex describes the origins of the University since it was first promulgated in 1911, the way in which it has been constituted, proposed developments and the kind of administrative and teaching and research arrangements which are being made for students.

Courses leading to degrees in the Faculty of Arts and Social Studies will be organized and pursued in a a different way from that followed in other British universities. The Faculty will be divided not into departments concerned with particular academic disciplines but into schools of study where different specialisms will be studied in relation to each other.

In 1962–63 there will be three such schools—the School of Social Studies, the School of English and American Studies, and the School of European Studies. A School of Afro-Asian Studies will follow later. Each School will have a dean at its head. In addition, there will be professors and lecturers in particular subjects. The schools will not be super-departments, and the same subject may be studied in more than one school.

Details of the science curriculum are still under consideration. It is intended to provide degree courses which will prepare students not only for careers as professional scientists, in the academic world, in industry, and in Government scientific establishments, but also for careers as teachers, and in industrial, commercial and public administration. There will be a three-year course in natural science, leading to a classified honours degree. During this course students will study three subjects chosen from pure mathematics, applied mathematics, physics, chemistry, and biology. In addition, the curriculum will require some study (which will be examined) of the history of science and of the impact of science on society.

For a limited number of selected students, there will be a second honours course lasting a year, in which one science only will be studied, and a successful candidate will be awarded classified honours in that subject. The student who has completed this further course will thus have a specialized knowledge of a single science, and some understanding of the social sciences.

There will also be a course lasting three years in engineering science. This will be a general course; it is not intended to provide specialized courses in civil, mechanical or electrical engineering.

During the years 1962–63 and 1963–64, only the subjects mathematics (pure and applied), physics and chemistry will be offered. It is hoped to begin courses in engineering in 1964 and in biology in 1965.

* The University of Sussex. Prospectus, 1961–62. Pp. 26 + 2 plates. (Brighton: The University of Sussex, 1961.).

METABOLISM OF THE HUMAN RED BLOOD CELL

SOME aspects of the metabolism of free fatty acids by the human red blood cell *in vivo* have been studied by Dr. D. Mendelsohn of the Department of Pathology and Microbiology, University of the Witwatersrand, Johannesburg (*S. Afric. J. Med. Sci.*, 20; Nos. 1 and 2; July 1961). Following the oral ingestion of glucose by a normal subject, an increase in erythrocyte-free fatty acid concentration occurs, reaching a maximum in 1–1½ hr. In contrast, the diabetic patient shows a much smaller rise in red cell-free fatty acid during a glucose tolerance test; in many diabetic cases no increase could be detected at all. This defect in the diabetic erythrocyte could be corrected by the intravenous administration of insulin during the performance of a carbohydrate tolerance test.

Although the findings do not permit any definite conclusions to be drawn, it is suggested that the human red blood cell is able to synthesize lipid from carbohydrate *in vivo*. This faculty seems to be impaired in the human diabetic erythrocyte.

Mendelsohn has also examined the interrelationships of carbohydrate and lipid metabolism in the human erythrocyte *in vitro*; all the findings observed when investigating the metabolism of lipids by the human red blood cell *in vivo* have been reproduced *in vitro*. The results of the investigation suggest that under certain *in vitro* conditions, that is, in a phosphate buffer containing glucose and brilliant cresyl blue or methylene blue, the mature human red blood cell is able to synthesize fat from carbohydrate. Any exchange of fat between red cell and serum which might have been expected to cause a rise in erythrocyte lipid-levels has been eliminated by incubating the cells in phosphate buffer instead of serum. The fact that normal red cells showed an increase in total esterified fatty acid together with free fatty acid indicates that the rise in cell-free fatty acid was not due to lipolysis of complex lipids with subsequent release of fatty acids.

A much less noticeable rise in the level of free fatty acids was apparent in the erythrocyte of an insulin-deprived diabetic patient when compared with a normal patient. Addition of insulin to the diabetic red cell suspension corrected this deficiency. It is suggested that under the *in vitro* conditions described, the mature human erythrocyte appears to be able to synthesize fat from carbohydrate whereas in the diabetic red cell this faculty is markedly impaired. Mendelsohn has also examined some of the mechanisms which might be responsible for the abnormal behaviour of the diabetic erythrocyte.

UNIVERSITY OF MICHIGAN LIBRARIES

XUM

STUDENT MENTAL HEALTH

IF one asks an examiner of the old-fashioned, bluff, extraverted type why students fail in their examinations he will possibly reply: "Because they are insufficiently prepared". No doubt he will be content to leave it at that, but if one continues the Socratic questioning and demands: "Why are they insufficiently prepared?" one will be told: "Because they haven't worked enough". But this is not the complete answer, even supposing that one is told that their lack of work depends on a poor personality, laziness, or something of the sort.

These students who pose such problems are, as Dr. Malleson pointed out at a conference on the mental health of students recently held in London*, those who have surmounted the difficult hurdles of the 'eleven plus', the Common Entrance, the General Certificate of Education examinations, and so on in the past. Why have they failed at the university?

The papers read at the conference suggest that there is no simple answer. Although the student may have been successful at school, there are psychological difficulties associated with university life which may interfere with his work. These range from home-sickness, if he lives in a hostel, or domestic unrest associated with parental quarrelling, if he lives at home to such grave psychological illnesses as schizophrenia, depression and the severe crippling neuroses.

Student failure may mean that a valuable young man gives up further education and takes up work inferior to his intellectual capacity. It means, moreover, a great deal of expense to the nation. It is estimated that a student costs about £700 a year to educate at a university. If he gives up his academic career, as most students that fail do, after a year and a half, he has wasted at least a thousand pounds of public money. When one considers the number of students the loss is potentially enormous.

What is to be done about it? The answer lies, to some extent, in the work of the psychiatric advisers, who have the opportunity of examining students complaining of psychological symptoms. Many universities have such a unit and, as Dr. Malleson emphasizes, if only four students a year are saved from giving up their academic careers in a year the adviser has earned his keep.

Such an advisory unit is not primarily to treat nervous or mental illness, although one of its functions is early diagnosis, but to help the much larger number of students to overcome the usual adolescent difficulties. Among those who complain of comparative trivialities the more sinister illnesses are found, and even these, if discovered early, can sometimes be helped.

The time has passed when students should be thrown ruthlessly into university life to sink or swim. Such a policy may have sounded bold and manly, but many young men with otherwise a fine intellectual future have sunk; have worried over a triviality until they have broken down or taken their lives uselessly.

Probably all those who are student advisers will agree that we do not know everything regarding the problems of academic life. It is one of the advantages of an advisory unit that there is an enormous amount of material for research purposes, and, since the student is a picked man, derived from an intellectual *élite*. Ultimately this work should throw a valuable light not only on student problems but also on mental health in general. CLIFFORD ALLEN

* World University Service. Student Mental Health: Report of a Conference held at Campbell Hall, London, 8th–9th April, 1961. Pp. 44. (London: World University Service, 59 Gloucester Place, 1961.) 2*s.* 6*d.*

TRAWL FISHING IN THE TROPICAL ATLANTIC

Catch-rates and Composition

IT is notoriously difficult to assess relative productivity between trawl fisheries in different parts of the world because of the inherent differences in fishing power of the vessels operating each fishery. Though it may be possible[1] to apply factors to convert the catch-rates of vessels of similar type, but of different size, to a standard form, this is generally only practicable within a limited geographical area. The investigations of the Colonial Office vessel, *Cape St. Mary*, off West Africa in 1952–57 and off South America in 1957–58, working with the same officers and gear on both sides of the Atlantic, offer an opportunity to compare the standing crop, in terms of catch-rate, of the demersal fish of the two areas. Mitchell and McConnell[2] have described their investigations off Georgetown on the Guiana coast, and an account of the work off West Africa is now in preparation[3]. Salzen[4] has described some early results from Ghana with the same vessel, though with a different crew.

The *Cape St. Mary* was a 100-ft. (between perpendiculars) steel trawler of rather typical North Sea design, of 238 gross tons and with 385-h.p. Diesel engine; details of her construction and gear are given in ref. 2. The trawls, in both areas, were of the Peter Carey type, 72 ft. on the head-rope, and were normally worked with 25-fathom bridles.

In the silty water and on the muddy deposits of those parts of the tropical Atlantic coast-line where there is heavy run-off of surface water from the land, there are few, if any, reef corals, and the ground is suitable for trawling; the demersal fish fauna is superficially similar on both coasts, comprising a large number of species, relative to temperate faunas, principally of Sciaenidae, Drepanidae, Pristipomatidae, Ariidae and Cynoglossidae, off West Africa, and of Sciaenidae, Pomadasydae, Gerridae and Ariidae off British Guiana. On harder, sandy deposits the fauna comprises principally Sparidae, Lethrinidae and Lutjanidae off West Africa, and Serranidae and Lutjanidae off British Guiana. However, the percentage composition of the catch is rather different off West Africa and off that part of South America (Table 1) which was investigated.

There appears to be a much greater dominance of Sciaenidae in the British Guiana catches than in those

Table 1. Percentage Composition of Commercial Landings at Four Tropical West African Ports, from Various Sources, and of the *Cape St. Mary* Landings at Georgetown, British Guiana

	Free-town	Abidjan	Takoradi	Lagos	George-town
Sciaenidae	34	21	44	25	67
Polynemidae	15	14	13	14	<1
Drepanidae (or Ephippidae	14	3	3	2	<1
Ariidae	11	2	—	14	4
Trygonidae	2	5	18	8	<1

from the African equatorial regions (Table 1) of comparable latitudes. In this respect the Guianian fisheries appear to resemble more closely those of Mauretania and Angola, to the north and south of the African equatorial zone, where a single species (*Sciaenia aquila*) dominates the landings, the more so since a single species (*Micropogon furnieri*) constituted up to 43 per cent of the total landings in Guiana.

In the final analysis of the catch-rates of the *Cape St. Mary* off West Africa[2], it was convenient to divide the data according to the fauna comprising each catch: a Sciaenid fauna on soft deposits down to the level of the thermocline at about 55–65 m.; a Sparid fauna on sand and shell-sand down to 90–110 m., and on mud between the thermocline and 110 m.; and a deep fauna, mainly of small, useless species, from 110 to 450 m., in which elements of the semi-abyssal continental slope fauna occurred. Results in ref. 2 are expressed in relation to deposit and depth but can readily be re-arranged to conform to the West African results, since a similar division of the fauna by deposits was recognized in that work.

Table 2. Catch-rates (kgm./hr.) of the *Cape St. Mary* on Four Sectors of the West African Continental Shelf between 13° W. and 17° W. and on the South American Continental Shelf between 7° N. and 8° N.

	Sulima	Free-town	Conakry	Gambia	George-town
Sciaenids, etc.	183	195	295	178	187
Sparids, or Serranids, etc.	21	53	53	130	59
Deep fauna	14	—	43	90	7·5

The results (Table 2) show that the catch-rates off West Africa and South America are very similar, with a single significant anomaly; this is the high catch-rate of snapper off Gambia. This region lies at the northern extremity of the equatorial zone on the West African continental shelf, near the limit of the tropical Sciaenid fauna and is characterized by a rich fishery for large snappers (*Dentex*, *Lethrinus*, etc.) which, furthermore, extends much deeper—at least to the continental edge—than does the tropical Sparid fauna farther to the south. A similar situation occurs at the southern end of the equatorial region in West Africa, to the South of Cape Lopez.

R. H. Lowe
(McConnell)

Fisheries Division,
Department of Agriculture,
Georgetown,
British Guiana.

Alan R. Longhurst

Preliminary Bionomic Data from West African Fisheries

The determination of bionomic parameters in tropical fisheries is a matter of some difficulty, due particularly to the lack of seasonal or periodic changes in the environment, which renders useless many of the classical techniques in temperate and boreal seas[5]. It has been shown[6] that demersal fish off Sierra Leone have, in general, no periodic cycle of feeding activity during the year, and it is known from general fisheries investigations there that many species are reproductively active throughout the year, juveniles of a few centimetres long being taken in the trawl debris in most months.

However, recent investigations at Freetown[3] have shown that some species—fortunately including some of the more important commercial species—do, in fact, have a period of peak breeding activity which appears to have an annual periodicity; it has been possible to use the annual peak recruitment to the trawl catches of juvenile fish, and their subsequent growth, to determine rates of growth and mortality, using Petersen's technique.

This evidence is derived from the inspection of the monthly length frequency distributions taken from trawling data—from commercial, exploratory and experimental trawling; the technique depends for its success on a number of factors: that a single population can be sampled monthly, or more frequently, over a period of years; that the samples should be taken with a very small-meshed net so that the 50 per cent selection point of the net falls on fish of not more than six months old; that the juveniles should be present on the same grounds as the adults, without ecological separation and, finally, that there should be no significant difference in size between the sexes. It appears that Sciaenidae and Drepanidae are the most likely families to fulfil all these requirements, and give the most satisfactory estimates of growth- and mortality-rates of the species investigated.

The results obtained for eight demersal species are set out in Table 3 in terms of length-for-age data.

Table 3. Length-for-age Data; Lengths expressed as Total Length (Snout to Tip of Longest Caudal) in cm. Doubtful estimates in parenthesis

	I	II	III	IV	V	VI
Pseudotolithus elongatus	15·0	24·5	31·5	36·0	39·2	41·2
Pseudotolithus senegalensis	(15·0)	(28·0)	(42·0)	—	—	—
Drepane africana	16·0	23·0	25·5	—	—	—
Chaetodipterus lippei	15·5	24·0	—	—	—	—
Galeoides decadactylus	11·0	19·1	26·3	34·0	—	—
Pentanemus quinquarius	8·5	15·5	20·5	31·0	—	—
Pagrus ehrenbergi	(18·0)	(32·0)	—	—	—	—
Pristipoma jubelini	(15·0)	(22·0)	(30·0)	—	—	—

In a single species, *Pseudotolithus elongatus* (=*Corvina nigrita*), the data sufficed for a more detailed analysis than is included in Table 3. This is an abundant species in the estuarine and inshore catches, and the monthly length frequency distributions showed very satisfactory progression of peaks, month by month. There was an annual peak of recruitment to the catches in June and July of small fish about 5 cm. long which appeared to be the result of a spawning peak during the dry season at the turn of the year, confirmed by evidence from the gonadial cycle.

From an investigation of the length/weight relationship of the species it is possible to derive an estimate of both relative and instantaneous growth in weight, and this is shown in Table 4.

Table 4. *Pseudotolithus elongatus*; Growth in Length and Weight (h = relative, g = instantaneous growth in weight)

Year	Length (cm.)	Weight (gm.)	h	g
I	15·0	22·0	—	—
II	24·5	109·0	3·95	1·60
III	31·5	222·0	1·03	0·71
IV	36·0	337·0	0·52	0·42
V	39·2	403·0	0·19	0·17
VI	41·2	439·0	0·09	0·09

From dissections of the length frequency distributions of a large sample taken in November 1959, with a very fine-meshed net, it was possible to make an estimate of the relative numbers of individuals in

UNIVERSITY OF MIAMI LIBRARIES

XUM

Table 5. *Pseudotolithus elongatus*: Instantaneous Mortality-Rate (*i*), Annual Mortality-Rate (*a*), and Annual Survival-Rate (*S*); Biomass in kgm. of the Survivors of 1,000 Fish at Year I (*P*)

Year	Log *n* − log *n* + 1	*i*	*a*	*S*	*P*
I	—	—	—	—	11·5
II	0·89	2·049	0·87	0·13	12·9
III	0·63	1·450	0·76	0·24	12·1
IV	0·63	1·450	0·76	0·24	2·2
V	0·43	0·990	0·62	0·38	1·0

each year-class and from these estimates, using Ricker's exponential tables[7] to make an estimate of mortality-rates. These are shown in Table 5.

From these estimations of mortality and from the length/weight relationship it is possible to estimate the relative biomass of the survivors year by year of a single-year class (Table 5). It is clear that in this species the relative weight contributed to the population by fish of more than 3 yr. old is relatively very small, and it is clearly desirable that exploitation should begin before this time. In fact, an estimate was made of the composition of the commercial landings at Freetown of this species and showed that 97·8 per cent of the individual fish landed were in their second or third year of life, and only 1·9 per cent were older fish.

In general, the tropical West African demersal fisheries appear to depend on only a relatively small number of year-classes of the constituent species, and this fact may be expected to have important consequences in any management policy—variations in the brood-strength of a species from year to year, for example, may be expected to have a far greater effect on the availability of a fish than is the case in a cold-water species, where the landings may depend on 10 or 20 yr.-classes—and this may, in part, be an explanation of the puzzling fluctuations in abundance which are so characteristic of fisheries in this part of the world.

The evidence on which these data are based will be published in full in the Colonial Office Fisheries Publications in due course.

Alan R. Longhurst

Federal Fisheries Service,
P.M.B. 2529, Lagos,
Nigeria.

[1] Beverton, R. J. H., and Holt, S. J., *Fish. Invest.* (2), **19**, 1 (1957).
[2] Mitchell, W. T., and McConnell, R. H., *Bull. Fish. Division, Department Agric. B.G.* (2), 1 (1959).
[3] Longhurst, A. R., *Fish. Pub. Colonial Office* (in the press).
[4] Salzen, E. A., *Nature*, **178**, 1105 (1956).
[5] Longhurst, A. R., *CSA Specialists Meeting, Monrovia*, **2**, 2 (1960).
[6] Longhurst, A. R., *J. Anim. Ecol.*, **26**, 369 (1957).
[7] Ricker, W. E., *Bull. Fish Res. Bd. Canada*, 119 (1958).

A NEW APPLICATION OF THE FARADAY MAGNETO-OPTICAL EFFECT FOR DIAGNOSTIC MEASUREMENTS OF TRANSIENT MAGNETIC FIELDS

By R. D. MEDFORD, W. H. W. FLETCHER, J. D. HERBERT and A. L. T. POWELL

Atomic Weapons Research Establishment, Foulness, Southend on Sea

EXTENSIVE use has been made of high-speed photography in plasma research but only to record plasma geometry, brightness, and the presence of instabilities and shock-waves. We now describe a new technique for recording time variations in a longitudinal magnetic field which makes use of the Faraday magneto-optical effect, uses simple inexpensive apparatus and is easily incorporated into experiments already using streak camera observations.

In an earlier paper[1] we described methods for superimposing streak (distance-time) and optical interference fringe photographs on the same emulsion. By combining this and the new technique it should be possible to determine geometry, space-time distribution of electron number density in a high-density plasma (about 10^{17} cm.$^{-3}$), and line-integrated variations of magnetic field along the line of sight with one streak camera.

The magnetic field inside a plasma is usually determined from magnetic probe readings, but such readings are limited, since the physical presence of the probe introduces impurities and results in a perturbation of the plasma. Even when the probe readings are only taken as valid up to the time when the plasma hits the probe, the problems of providing screening against radiation and protecting the recording oscilloscope from extraneous Earth currents is formidable. The lifetime of plasma probes is also short.

An interesting technique has been described in ref. 2; it provides an independent method of measuring the longitudinal magnetic field inside a plasma without inserting probes and also gives the electron density. But the method is only applicable to plasmas having a sufficiently low electron density to give a frequency below the propagating microwave frequency and a magnetic field strength sufficiently large to give a measurable ratio of electron Larmor frequency to propagating frequency (w_L/w). In our experiments we work in the optical region with wave-lengths of 5×10^{-5} cm. and magnetic fields of about 10^4 œrsted. This means that we cannot use the Consoli-Dagai technique, since $(w_L/w) \simeq 10^{-5}$. However, this low ratio of (w_L/w) means that the electron density can be more accurately determined from the optical fringe pattern[3] and when the electron density distribution is known it is relatively easy to compute the associated magnetic field distribution inside the plasma[1]. The magnetic field distribution determined in this way is, of course, a function of the magnetic field strength at the plasma boundary, which must be determined independently. We determine the magnetic field at the boundary by making use of the Faraday magneto-optical effect in the walls of the plasma discharge tube.

The experiment described here is our first experiment using the magneto-optical technique and is designed to measure the driving magnetic field inside a theta piece[4–6]. The cylindrical discharge tube was made of extra dense flint glass, Chance number 70030, and has a Verdet's constant of about 0·09 where the Faraday effect is given by:

$$\theta = rHL$$

Fig. 1. Theta pinch at 1 mm. ambient pressure

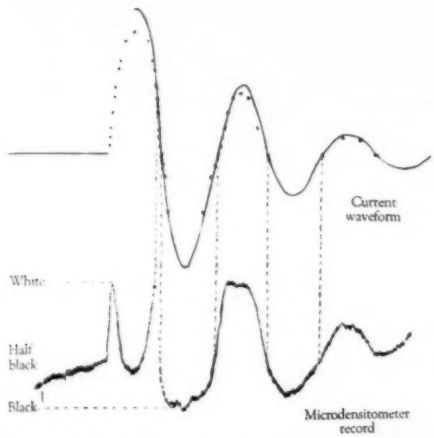


Fig. 2. Theta pinch waveforms. ×, from densitometer record

θ is the rotation of the plane of polarization in minutes; H is the strength of the magnetic field in œrsted directed along the optical axis; L is the path-length in cm.; r is Verdet's constant and is given by the old classical theory[7] as:

$$r = \frac{2\pi N e^2 w^2}{c^2 n(f - mw^2)^2}$$

where N is the number of electrons/unit volume; e and m are electron charge and mass; c is the speed of light; $2\pi(m/f)^{1/2}$ is the electron 'free period'; w is the angular frequency of propagation; n is the refractive index in the absence of magnetic field.

We have repeated the derivation of r allowing for the fact that H has a Fourier spectrum in the transient case and for an H component of angular frequency Ω we find that the frequency components of H modulate Verdet's constant so that w must be replaced everywhere by $(\Omega + w)$. Fortunately, in our experiment $(\Omega/w) \ll 1$ and Verdet's constant is not appreciably modulated.

To measure the rotation of the plane of polarization we use a polarizer and an analyser to convert rotation into a change in intensity in the transmitted light. Since the intensity is proportional to the square of the cosine of the angle between the plane of polarization of the light incident on the analyser and the plane of polarization for maximum transmission through the analyser, the analyser and polarizer are set with their planes of polarization at 45° to give maximum sensitivity and easy determination of magnetic field direction. The external driving field is derived from the rotation of light in the cylindrical wall. The light is restricted to a sufficiently narrow band-width 5400–6400 Å. by the polarizer. At a suitable part of the camera sweep the light source is pulsed and a short time afterwards the theta pinch is initiated. Light from the light source is also allowed to enter the camera without passing through the discharge tube to provide a light-intensity datum.

Fig. 1 shows a streak camera photograph of a theta pinch in deuterium, ambient pressure 1 mm. of mercury fed by a 6 μF. condenser charged to 9 kV. Reading the photograph from top to bottom the first track is of light from the light source direct, the second track is of the polarized light passed through the discharge tube and shows clearly the modulation produced by the theta pinch magnetic field.

Fig. 2 shows the current in the theta piece monitored across a small series resistance together with a microdensitometer reading of the second track in Fig. 1. The vertical lines show the correlation between points of zero magnetic field and half-black light intensity points. It will be noted that there is an extra half-black point corresponding to the large rotation produced by the high initial magnetic field. To calibrate the apparatus the theta piece was evacuated and fed with current from a known condenser, thus providing a damped oscillation of the magnetic field.

Fig. 3 shows the associated streak photograph and Fig. 4 the associated current and microdensitometer waveforms. Once again there is an extra half-black point and two humps, which provide excellent self-calibration since they define a rotation of 45° in the discharge tube.

Using magnitudes of magnetic field defined by the damped current waveform and the first 45° point we find that the Verdet's constant for the discharge tube glass was 0·094 min./œrsted cm. and using the calibration waveforms we have superimposed on the current waveform in Fig. 2 the points obtained from the microdensitometer trace in Fig. 2. It will be seen that the correlation is excellent and that the optical method fills in the initial rise that was too fast to be recorded by the current oscilloscope. There was no need in this experiment to use the light-intensity datum trace in the interpretation of the microdensitometer traces shown in Figs. 2 and 4, since we were fortunate in having a special high-intensity lamp developed by Dr. Gabriel of the Atomic Weapons

Fig. 3. Calibration streak photograph

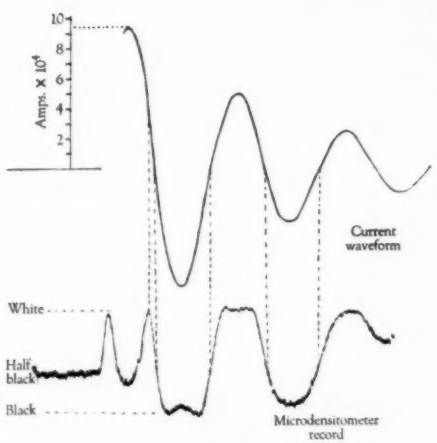


Fig. 4. Calibration waveforms

UNIVERSITY OF MICHIGAN LIBRARIES

XUM

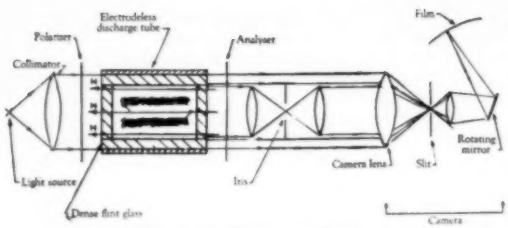


Fig. 5. Proposed optical system

Fig. 6. Streak-interferogram at 1 mm. ambient pressure

Research Establishment[3] that gives a light pulse of about 10 μsec. duration which is remarkably constant. However, we feel that the datum trace would have corrected for a lamp of inferior type. It is also fortuitous that the polarizer and analyser make the light a sufficiently narrow band for the purpose of the experiment and high-quality interference filters are not required.

Fig. 5 is a schematic diagram of our next experiment and is included to show how the technique may be extended to measure not only the driving magnetic field but also the trapped magnetic field in a theta pinch. The diagram shows how the end windows of a pill-box type discharge tube can be used to measure the trapped field on the axis which is expected to have small divergence at the ends of the theta piece. The main feature of the optics is an iris diaphragm placed between a small supplementary lens and the exit lens feeding the light from the centre of the theta piece to the camera. By controlling the diaphragm aperture the transmitted light through the centre will have a good 'signal-noise' for measurement of trapped field to be made even when the plasma fills the whole cross-section[1].

Fig. 6 is a streak camera record of a theta piece with interference fringes superimposed and is added for sake of completeness so that the reader can visualize a composite picture showing fringe shift and trapped and driving magnetic fields. We wish to thank Beryl Richardson for her invaluable assistance in the experiment. We are also indebted to Dr. Gabriel for supplying the special light source and the Director of the Atomic Weapons Research Establishment for permission to publish this article.

[1] Wright, J. K., Medford, R. D., Hunt, A. G., and Herbert, J. D., *Proc. Phys. Soc.* (to be published).
[2] Consoli, T., and Dagal, M., *J. Nucl. Energy*, Part C, **3**, 115 (1961).
[3] Medford, R. D., Powell, A. L. T., Hunt, A. G., and Wright, J. K., *Proc. Fifth Intern. Conf. on Ionization Phenomena in Gases*, Munich, 1961 (to be published).
[4] Wright, J. K., Eeles, W. T., and Herbert, J. D., *Nature*, **183**, 1665 (1959).
[5] Medford, R. D., Powell, A. L. T., Herbert, J. D., Pottinger, R. C., and Wright, J. K., *Nature*, **186**, 706 (1960).
[6] Glasstone, S., and Lovberg, R. H., *Controlled Thermonuclear Reactions*, 415 (Van Nostrand).
[7] Houston, *A Treatise on Light*, 477 (Longmans).
[8] Gabriel, A., *J. Sci. Instruments*, **37** (1960).

SOME PHYSICO-CHEMICAL PROPERTIES OF PARACRYSTALLINE SPHERULITES OF BIOLOGICAL ORIGIN

By Dr. G. T. STEWART

Medical Research Council Laboratories, Carshalton, Surrey

THE occurrence of lipoid in a mesophase in certain mammalian tissues has already been reported[1]. Matter in this form possesses paracrystalline or liquid crystalline properties whereby the molecular compactness and regular orientation, characteristic of a true crystal, is preserved when, in a given thermal range, liquid mobility is demonstrable. There are theoretical reasons for believing[2] that a mesophase of this kind is ideally suited for protoplasmic structure; in the case of certain lipoid complexes, it has now been shown[3] that a mesophase with characteristic liquid crystalline properties exists, naturally, in specialized cells of the ovary, adrenal and other tissues, though not in depot fat; pathologically, a similar mesophase may be present in plasma lipoprotein and in the soft parts of atheromatous deposits in arteries. Under experimental conditions, similar lipoid complexes can be produced by pulsating the lipoprotein fraction against normal arterial intima at physiological pressures[4].

Known for years to physicists and physical chemists[5], the mesomorphic form of matter has not been extensively studied in biological material, nor in relation to disease. The following report therefore describes further some of the properties of the spherulitic liquid crystals observed in various forms of lipoid mesophase in tissue.

Material for study was obtained by making squashed preparations of the appropriate tissues in microscopic films, or, when bulkier yields were necessary, by homogenizing the tissue in water. For general use, an abundant yield of paracrystalline substance was readily obtained by homogenizing segments of liver, taken by biopsy or at autopsy, from rabbits fed with 0·5–1·0 per cent cholesterol for three months or more. From this homogenate, intracellular and cell-free lipoid were separated by centrifuging, and independently studied in aqueous or other suspensions. More exact details of technique are described elsewhere[3], and paracrystalline substance has also been studied in lipæmic plasmas, resected atheromatous vessels in patients, corpora lutea in surgically excised ovaries, and other sources. Examined in microscopic films under polarized light, fresh preparations of the tissues mentioned show abundant birefringent particles intra- and extracellularly including, in the soft lipoid components, spherical droplets 0·5–2μ in diameter. These aniso-

tropic droplets (spherulites) have firm but not rigid, uniformly spherical outlines at 4–25° C.; each droplet contains a polarization cross with arms parallel to the planes of polarization. When the film is rotated, the arms do not change direction or undergo extinction; when the analyser is rotated, the cross is extinguished at 180° intervals. The sign of birefringence is positive and, with the polarizer only *in situ*, a faint interference pattern may still be seen. These droplets are therefore liquid crystals of spherulitic structure, without surface attachments to the suspending liquid or, when intracellular, to the cytoplasm.

On a heated stage at 30°–37°, the spherulites tend to increase in size by limited confluence and are then deformed in outline when compressed; the polarization figure loses its symmetry but retains a roughly cruciate pattern and a positive sign of birefringence. At 37°–42°, liquid flow is present, with birefringence; at higher temperatures, a true liquid, transparent, isotropic melt results. From this, a mesophase with considerable liquid crystalline growth of spherulites and an orderly arrangement of polarization colours re-forms on cooling. At 0° C. or below, the spherulitic structure is destroyed, the degree of destruction being more or less proportional to the rate and intensity of cooling; placed for a few seconds in liquid nitrogen, the liquid crystals are instantaneously and permanently destroyed, with liberation of free cholesterol in amorphous or true crystalline form, true crystals of fatty acids and other indeterminate particles. Individual spherulites show paracrystalline growth when kept for some hours in water or salt solutions, but not in glycerol. They are destroyed by organic solvents and dehydration. In white light, they are greenish and refractile; the larger spherulites may show a weak reaction with sudan dyes; when treated with benzpyrene[6], they show faint blue fluorescence in ultra-violet light.

These properties of the spherulitic component of this lipoid mesophase indicate that the constituent molecules are radially arranged, unstable to changes in temperature, dehydration and organic solvents, but stable at 4°–37° and, in this state, penetrable by water and triglycerides. The spherulitic structure is, however, resistant to disruption by various other physical agencies, including ionic and non-ionic detergents, lipase, rat-liver esterase, trypsin and ultracentrifuging in salt solutions at 80,000*g*. When subjected to ultrasonic vibrations (20 kc./s.), no structural change can be detected in 30 sec. but disintegration begins thereafter and is complete in 3–5 min. γ-Radiation up to 5 M.rads from a cobalt-60 source produces no change nor does irradiation with 4-MeV. electrons from a linear accelerator.

It is known[7] that some of the lipoid in abnormal, hyperlipæmic plasma is resistant to the action of clearing factor, and this may also be related to the presence of a stable mesophase. When heparin, or a comparable sulphated polysaccharide, is injected intravenously into a patient with essential hyperlipæmia (or a cholesterol-fed rabbit) with an abnormal, low-density β-lipoprotein plasma component, the classical clearing effect is often not obtained. This anomaly is due to the abnormal β-lipoprotein which resists, or may even inhibit[8], the action of lipoprotein lipase, considered by some authorities to be the active clearing factor[9]. If the abnormal lipoprotein is then separated by centrifugal flotation from the uncleared plasma and examined at 22°–37° under polarized light, much of it is present in the form of a spherulitic mesophase, similar in properties to that already described in atheromatous and other tissues. This abnormal lipoprotein complex can therefore exist within the plasma colloid as a mesophase, more stable than the chylomicron emulsion of normal lipæmia, and immune to one of the principal physiological clearing mechanisms. This is the form of lipoprotein which, when pulsated against excised arterial segments, produces atheroma-like deposits in a similar lipoid mesophase[4]. It is therefore possible that the presence in circulating blood of this form (*Sf* 12–20) of low-density lipoprotein is of direct causal importance in atherosclerosis.

Histochemical and chromatographic work[3] has shown that cholesterol esters and other lipids, as well as protein and water, are probably present in spherulites of the type described. These constituents must therefore be linked in an amphiphilic system within a molecular structure which, at body temperature, is loosely radial and perhaps helical. The physicochemical possibilities inherent in such systems have been made explicit in recent work[10,11] on phase-equilibria of soaps, water and sterols; it is highly probable that, in cytology as in physics, some of the particles which used to be called fatty micelles should now be regarded as paracrystalline complexes with this kind of structure which makes them amenable to incorporation in protoplasm while preserving the molecular orientation and penetrability to other lipoids and water, necessary for reactivity. Apart from this functional aspect, the importance of a liquid crystalline system in polypeptide solutions[12], and of spherulitic texture in polyethylene fibres[13] suggests, by analogy, that the paracrystalline form may be equally essential as a structural mechanism in certain cells and tissues, especially where complex macromolecules of lipoprotein and steroids are involved.

It is a basic property of liquid crystals that their molecules are held together by forces which, though complex, are weaker than those of true crystals. In the case of the liquid crystals described here, the weakness is revealed by the effects of temperature and organic solvents, but the bonds are remarkably resistant to agencies such as ionizing radiation, ultrasonic vibrations and lipolysis, whether by enzymes or detergents, which readily disrupt lipoid complexes in fine emulsions and cell membranes. The implications of this peculiar stability, in terms of energy values, are beyond the scope of this article, but it is perhaps clear that a study of this question, coupled with a crystallographic analysis of structure, should be of value in solving a fundamental and largely unexplored problem in molecular biology.

The irradiation experiments were carried out at the Research Laboratory of the United Kingdom Atomic Energy Authority at Wantage, Berks., in collaboration with Dr. D. B. Powell.

[1] Stewart, G. T., *Nature*, **183**, 873 (1959).
[2] Bernal, J. D., *Trans. Faraday Soc.*, **29**, 1032 (1933).
[3] Stewart, G. T., *J. Path. Bact.* (in the press).
[4] Stewart, G. T., *Brit. J. Exp. Path.*, **41**, 389 (1960).
[5] *Disc. Faraday Soc., Liquid Crystals and Anisotropic Melts* (1933).
[6] Berg, N. O., *Acta Path. Scand.*, Supp. 90 (1951).
[7] Stewart, G. T., *Brit. J. Exp. Path.*, **39**, 109 (1958).
[8] French, J. E., Robinson, D. S., and Harris, P. H., *Quart. J. Exp. Physiol.*, **40**, 320 (1955).
[9] Korn, E. D., *J. Biol. Chem.*, **215**, 15 (1955).
[10] Luzzati, V., Mustacchi, H., and Skoulios, A., *Disc. Faraday Soc.*, No. 25, 43 (1958).
[11] Lawrence, A. S. C., *Disc. Faraday Soc.*, No. 25, 51 (1958).
[12] Robinson, C., Ward, J. C., and Beevers, R. B., *Disc. Faraday Soc.*, No. 25, 29 (1958).
[13] Keller, A., and O'Connor, A., *Disc. Faraday Soc.*, No. 25, 114 (1958

UNIVERSITY OF MICHIGAN LIBRARIES

XUM

KINETICS OF REACTION OF THE DECOMPOSITION OF LEAD AZIDE

By DR. M. STAMMLER, J. E. ABEL and DR. R. C. LING
Picatinny Arsenal, Dover, New Jersey

IT has been reported[1,2] that the decomposition of inorganic azides begins at the crystal surface and that the crystal is soon covered with a thin film of metal formed by the reaction:

$$2MeN_3 \rightarrow 2Me + 3N_2 + X \text{ kcal. } (Me = \text{metal ion})$$

This reaction has been investigated extensively in the case of silver azide. Moskovich and Aleksandrovitch[3] reported the formation of lead atoms at active surface regions during the initial stage of decomposition of α-PbN_6. These lead atoms then act as crystallization centres and cause an autocatalytic acceleration of the decomposition which is accompanied by the accelerated formation of a second solid-phase Pb-PbN_6. We have investigated in detail the reaction kinetics involved in this decomposition of α-lead azide.

In ref. 4 the isothermal decomposition of α-lead azide at 150°, 175° and 200° C. in air was described. It was found that at those temperatures the decomposition goes through at least two phases. One phase could be isolated and classified as a tetragonal deformed cubic structure but, unfortunately, the other phases could not be isolated. According to the chemical analysis of the compound isolated, oxygen could be present only to a small extent. These experiments did not show whether the oxygen of the air was important in the formation of these phases during the decomposition, and the question could then be raised as to whether they consisted of basic azides or other oxygen-containing compounds as had been reported in ref. 5. These authors reported that basic lead azides have been prepared by hydrolysis of PbN_6 with water, by reaction of PbN_6 with sodium hydroxide, and by precipitating PbN_6 from lead salt solutions via the addition of a mixture of NaN_3 and sodium hydroxide. Nine different basic lead azides, including three forms of $PbN_6.xPbO$, were identified using X-rays.

X-ray work on phase 3. It has been mentioned[4] that during the isothermal decomposition of α-lead azide at 175° C. in air the last stage of decomposition corresponds to a compound with an approximate formula of $(PbN_{2\cdot5}(OH)_n)$, herein called phase 3. (The nitrogen is probably present as azide nitrogen.) Additional calculations show that some weak X-ray reflexions, which could not be indexed as cubic and which have been ascribed to impurities resulting from other decomposition phases, belong to the structure of phase 3. The crystal structure of this compound is more appropriately considered as a tetragonal deformed cubic lattice. On this basis the following data have been calculated:

cell dimensions: $a_0 = 12{\cdot}25_4$ Å.; $c_0 = 11{\cdot}88_3$ Å.; $c_0/a_0 = 0{\cdot}9698$;
volume of the unit cell, $V_0 = 1784{\cdot}66$ Å.^{3};
density (pycnometric): $d_p = 5{\cdot}623$ gm./cm.^{3};
density (X-ray): $d_x = 5{\cdot}633$ gm./cm.^{3}.

Debye–Scherrer photographs and X-ray diffractograms of this compound showed the following systematic absences:

(hkl) - - - - - absent except $h + k = 2n$
$(h\bar{k}0)$ - - - - - absent except $h + k = 2n$
$(0kl)$ - - - - - absent except $k + l = 2n$
$(h0l)$
(hhl) - - - - - no conditions
$(h00)$ - - - - - no conditions

These extinction data make possible the assignment of the space-groups D_{3h}^{12}, $P4_2/nnm$ and D_{4h}^{7}, $P4/nmm$, with the first of these being the most probable. During about thirty experiments this structure was observed within 16·0–16·8 per cent loss in weight of the samples. This is approximately the range of homogeneity of this phase.

It is interesting to note that phase 3 can also be formed by heating α-lead azide at higher temperatures for shorter periods of time. For example, this compound was formed by heating α-lead azide at 360° C. for 3 min. Longer heating will result in a change in colour from yellow to orange and finally brown.

Decomposition in pure argon and oxygen atmospheres. To investigate the influence of oxygen during the isothermal decomposition of lead azide, experiments using thermogravimetric techniques were carried out at 175° C. in argon and oxygen. While the decomposition in air with 40 per cent relative humidity led to an average of 16·4 per cent loss in weight, experiments in dried argon and oxygen resulted in a 12 per cent loss at the same temperature (Fig. 1).

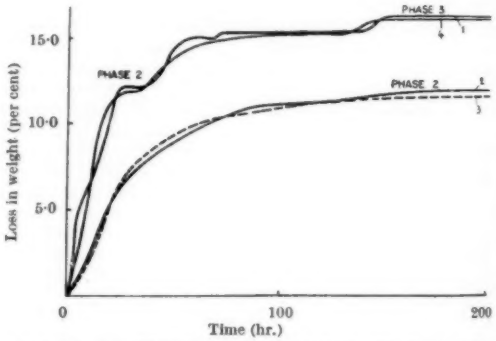


Fig. 1. Decomposition curves of lead azide in air with 40 per cent relative humidity (1); in dried argon (2); oxygen (3); and argon saturated with moisture at 26° C. (4)

It has been shown[4] that α-lead azide decomposes in air in several steps which must be considered as definite crystal phases. During decomposition at 175° C. the first step is reached when the sample has lost about 12 per cent in weight. However, according to the shape of the curve up to this step, it is possible that there is more than one reaction mechanism involved. Up to a 4·6 per cent loss in weight the slope of the curve, that is, the rate of evolution of nitrogen, is equal to 1·3 per cent/hr., whereas the rate of volatilization of nitrogen in the second

stage, up to about 9 per cent loss in weight, is 0·36 per cent/hr. and, up to 12 per cent loss in weight, about 0·5 per cent/hr. These steps can be seen in Fig. 1, which indicates a clear break in the curve at 4·6 per cent loss in weight which may be due to the formation of an intermediate state not shown in Fig. 6.

The decomposition does not follow this course if the experiment is run in dried argon and oxygen. While the first step in air containing 40 per cent relative humidity occurs at 175° with 12 per cent loss in weight after about 30 hr., the same result was obtained in dried argon and oxygen after about 100 hr. The decomposition in oxygen or argon follows a continuous curve and does not show the changes in the reaction-rate observed in air (Fig. 1). However, the X-ray diffraction patterns of the products formed in air at ambient conditions, in dried argon and in dry oxygen are the same. This indicates that, until this stage of decomposition, oxygen does not affect the reaction. It is very probable that the presence of moisture accelerates the decomposition reaction and causes at least a partial hydrolysis.

It is believed that subsequent decomposition (greater than 12 per cent loss in weight) is only possible in the presence of moisture. On a weight basis, the residue corresponding to the 12 per cent loss in weight may be identified as phase 2, $PbN_{3\cdot5}(OH)x$. This compound can be formed either by heating PbN_6 in air with moisture (relative humidity 40 per cent) at 175° C. for 30 hr. or by heating in oxygen or argon at the same temperature for 100 hr. As expected, phase 2 formed by these three experiments showed identical X-ray diffractograms. It is possible that even in the 'dried' argon and oxygen, traces of moisture are present which may contribute to the formation of his phase. In Fig. 2 the observed reflexions of this phase are compared with those of pure α-lead azide. Phase 2 seems to have a higher symmetry than the original α-lead azide but a lower symmetry than phase 3.

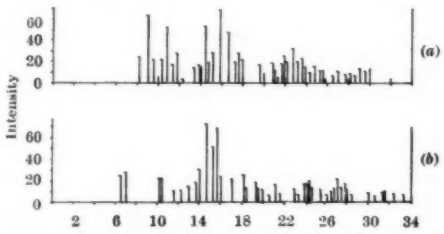


Fig. 2. X-ray reflexions of (*a*) α-lead azide; (*b*) phase 2

Apparently moisture affects the rate of decomposition, but the manner in which it accomplishes this is not fully understood at the present time. It is believed that small amounts of water vapour may hydrolyse the lead azide at elevated temperature[5]. If layers of lead were formed during decomposition, one would expect different results because such lead, being very reactive, would immediately form lead oxide in the presence of oxygen. Neither the reflexions of lead oxide nor those of metallic lead could be observed on the X-ray diffractograms of phase 2 obtained through decomposition in oxygen, argon or air. The formation of phase 3 could not be achieved at the given temperatures in pure argon and oxygen and it was concluded that a relatively high moisture content is necessary for the formation of this phase. (Phase 1, previously called α-phase[1], was observed only when heated at 150° C.) However, phase 3 was also obtained when α-PbN_6 was heated in argon which had been bubbled through water at 26° C.

Comparison of some properties of α-lead azide and phase 3. The ignition time of phase 3 was determined at several temperatures and compared with that of α-lead azide under the same conditions. The apparatus used for these experiments is shown schematically in Fig. 3.

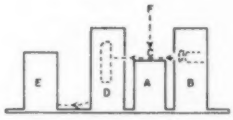


Fig. 3. Apparatus for measuring time for ignition. *A*, electrically heated plate; *B*, source of light; *C*, beam of light; *D*, photomultiplier tube; *E*, Hewlett-Packard timer; *F*, sample

The instrument consists essentially of a small electrically heated hot plate (*A*) with a centrally located iron-constantan thermocouple for determining its temperature and a photoelectric system for triggering the electronic timer. A 1/16-in. diameter light beam is focused across the centre of the hot plate (*A*) to a photomultiplier tube (*D*). When the hot plate is at the desired temperature, about 0·003 gm. of sample is dropped through the light beam (*C*) on the plate. The falling sample breaks the light beam and causes the photomultiplier tube to put out a pulse which starts the counter (*E*). The flash accompanying the ignition of the sample actuates the photomultiplier, which then stops the counter. The time until ignition is determined to 0·001 sec. and the temperature of the plate to ± 1° C.

The results so obtained for phase 3 and α-lead azide are plotted as temperature versus induction-time curves in Fig. 4. In both curves (4*a* and 4*b*) every point is the percentage fired out of twenty tests. The standard deviation of this method is about ± 5 per cent. An attempt was also made to calculate the activation energies of these compounds using the Semenov equation[7] and the plot of this is shown in Fig. 4*b*. The activation energies of phase 3 and PbN_6 were determined to be 80 and 100 kcal./mole, respectively. It should be mentioned that these activation energies are only apparent activation energies[6].

According to these results the compound $PbN_{3\cdot5}(OH)_n$ is less sensitive to ignition than the α-lead azide. It was noted that below 450° C. phase 3

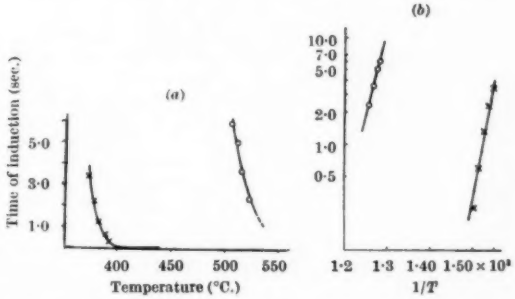


Fig. 4. (*a*) Temperature against time of induction. ×, α-lead azide; ○, phase 3. (*b*) time of induction against 1/*T*. ×, α-lead azide with energy of activation 100 ± 20 k.cal./mole; ○, phase 2 with energy of activation of 80 ± 20 k.cal/mole

UNIVERSITY OF MICHIGAN LIBRARIES

XUM

was found to undergo slow decomposition, but no ignition was observed at this temperature. On the other hand, the lowest ignition temperature for lead azide is about 370° C. and its ignition time at 400° C. is less than 0·1 sec.

To test the electrical sensitivity of phase 3, small 1/4-in. diameter plugs of insulating material were coated with a thin gold film to form an electrical bridge and a 0·06 gm. pellet of sample was pressed at 3,000 lb./sq. in. on the gold film. The resistance of the loaded plugs ranged from 1 to 3 ohms and their firing characteristics were determined by the discharge of a 1 μF. condenser at varying voltages. In Fig. 5 percentage fired is plotted against the input voltage. For comparison, the electrical sensitivity of lead azide is also included in Fig. 5. It is apparent that although less sensitive than the untreated lead azide, phase 3 can still be considered as an electrically sensitive material.

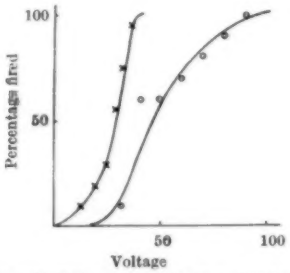


Fig. 5. Electrical initiation of α-lead azide (×) and of phase 3 (○)

Investigations with infra-red. The infra-red spectrum of phase 3 is slightly different from that of α-lead azide, although the main absorption band at 4·93μ occurs in both samples with the same intensity. In Table 1 the observed absorption bands of α-lead azide and phase 3 are listed. The pellets used for the infra-red analysis were prepared by pressing 200 mgm. KBr and 2 mgm. of PbN_6 or $PbN_{2\cdot5}(OH)_n$ at 15,000 lb./sq. in. The thickness of these pellets was approximately 1 mm.

The absorption of both samples was most intense at about 5·0μ. This band can best be ascribed to a stretching vibration of the N≡N triple bond. As indicated in Table 1, only slight differences between the two samples could be observed so far as their infra-red spectra were concerned. In the case of lead azide the most intense absorption band consists of two distinguishable peaks at 4·93μ and 5·05μ respectively, whereas with phase 3 there is only one peak which occurs at 4·95μ. Another absorption band of lead azide with a weaker intensity was observed at 7·55μ due to the symmetrical vibration of the azide group. In this region the decomposition product (phase 3) showed two absorption maxima at 7·15–7·20μ and 7·63μ. The first band is probably due to the N=N double bond while the peak at 7·63μ is possibly caused by an OH bending vibration. In the spectrum of both α-lead azide and its decomposition product, an absorption band at 2·95μ could be observed. However, the intensity of this band in the spectrum of phase 3 was found to be twice the intensity of the lead azide peak. This would suggest that hydrogen bonding is present in both cases. Although the samples had been dried very carefully to remove all moisture and water, the spectra showed that this band (2·95μ) was still present. It is very probable that the lead azide also contained

Table 1. Infra-red Spectra

α-Lead azide (PbN_6)			(Phase 3) $PbN_{\sim 2\cdot5}(OH)_n$		
Absorption band	Int.	Remarks	Absorption band	Int.	Remarks
2·95μ (3,400 cm.$^{-1}$)	4·5	OH bonding vibration	2·95μ (3,400 cm.$^{-1}$)	9·3	OH bonding vibration
4·30–4·35μ (2,325–2,300 cm.$^{-1}$)	2·0	hydrogen bond	4·30μ (2,325 cm.$^{-1}$)	2·5	hydrogen bond
4·93μ (2,030 cm.$^{-1}$	25·0	asymmetrical stretching of the azide group	4·95μ (2,030 cm.$^{-1}$)	24·0	stretching vibration of the N≡N triple bond
5·05μ (1,920 cm.$^{-1}$)	24·5				
6·10–6·20μ (1,640–1,610 cm.$^{-1}$)	3·5	N—H bending vibration	6·10–6·20μ (1,640–1,610 cm.$^{-1}$)	8·0	N—H bending vibration
			7·15–7·20μ (1,400–1,390 cm.$^{-1}$)	9·0	N=N bond
7·55μ (1,325 cm.$^{-1}$)	2·8	symmetrical stretching vibration of azide group			
			7·63μ 1,310 cm.$^{-1}$	6·5	OH bending vibration
			14·75μ 678 cm.$^{-1}$	5·0	not known

a small amount of bonded water which could not be removed by the normal drying procedure and which might be considered as a stabilizer (through OH-bonding) of these compounds. Apparently even heat treatment with decomposition of lead azide does not remove this water. For future investigations it would be of interest to prepare lead azide without using water as a solvent, if possible. Another absorption band was observed in both samples at approximately 6·10–6·20μ which may be attributed to the N—H bond. Again, the band due to phase 3 was twice as intense as that of untreated lead azide. Finally, a weak absorption band which occurred in both samples with about the same intensity could be observed at 4·30–4·35μ. This may be caused by hydrogen bonding in the condensed state. One cannot, however, arrive at a final conclusion based on these results at the present moment, but more information may be forthcoming if lead azide can be prepared in a non-aqueous solution.

Conclusions. Based on the results obtained thus far, it appears that the reaction mechanism during the thermal ignition of lead azide in air is dependent on the temperature and time to ignition. Ignition times of less than 0·5 sec. have a single mechanism which probably goes through an activated state by which the required energy for a chain reaction is delivered (mechanism I, Fig. 6). These activated centres have to reach a certain concentration in a short time-interval in order to deliver enough energy for ignition. If, on the other hand, the temperature is not high enough to form this threshold concentration (there may also be a threshold temperature) the available energy cannot be supplied to cause the chain reaction and the decomposition will proceed slowly through

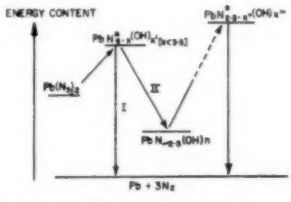


Fig. 6. Kinetics of decomposition of lead azide

the different phases (mechanism II, Fig. 6). It is possible, however, that within a certain range of temperature both mechanisms are competing with each other. For the slow decomposition, a hydrolysis reaction is assumed since the presence of oxygen during this decomposition had no effect on the hydrolysis reaction.

We thank Dr. J. V. R. Kaufman for his interest and encouragement during the course of this work, Mr. C. Campbell for the thermogravimetric work, Mr. J. Campisi for the X-ray photographs, and Dr. N. Paraskevopoulos for the infra-red spectra.

[1] Bowden, F. P., and Yoffe, A. D., *Fast Reaction in Solids* (Cambridge Univ. Press, 1958).
[2] Mitchel, J. W., *Rep. Prog. Phys.*, **20**, 433 (1957).
[3] Moskovich and Aleksandrovich, *J. Phys. Chem.* (U.S.S.R.), **6**, 1218 (1935).
[4] Stammler, M., Abel, J., and Kaufman, J. V. R., *Nature*, **185**, 456 (1960).
[5] Feitknecht, W., and Sahli, M., *Helv. Chem. Acta*, **37**, 1423 (1954).
[6] Reitzner, B., *PATR* (in the press).
[7] Semenov, N., *Chemical Kinetics and Chain Reaction* (Oxford, 1935).

PROBABILITY DISTRIBUTION ANALYSER PROGRAMMED FOR NEUROPHYSIOLOGICAL RESEARCH

By Dr. W. R. LEVICK, Prof. P. O. BISHOP and W. O. WILLIAMS
Brain Research Unit, Department of Physiology, University of Sydney
AND
Prof. D. G. LAMPARD
Division of Electrotechnology, Commonwealth Scientific and Industrial Research Organization

THE sensory side of the nervous system is basically 'noisy'. Information supplied by sensory stimulation is transmitted through the brain as patterns of nerve impulses. In the absence of stimulation, however, the trains of nerve impulses do not subside completely but are replaced by a 'spontaneous' discharge which may be quite as striking as the information-rich pattern. It is perhaps surprising that so little attention has been given to an understanding of the nature of this spontaneous discharge. There are, however, serious practical difficulties. Superficially this discharge of nerve impulses appears to be without any pattern. Apart from using devices which indicate the mean rate of the unit discharge, one must undertake a very large amount of tedious manual computational work to derive more comprehensive statistics and this has undoubtedly been responsible for the lack of interest in this phenomenon.

Recently a probability distribution analyser was described[1] based on the Hutchinson and Scarrott pulse-height analyser, used extensively in nuclear physics. It has been successfully applied to computational problems in the field of stochastic processes and noise. We have now programmed this instrument to derive the frequency distribution of intervals between successive impulses of the spontaneous (and light-evoked) discharge of single units sampled at various points in the visual system. The distribution is presented as an 'interval histogram'. While this work was in progress other workers have independently derived similar statistics from the auditory system[2].

The probability analyser is basically a cathode ray tube electrostatic memory of 64 channels with 15 binary digit capacity a channel. The voltage applied to its input terminal is ordinarily sampled at a fixed (relatively slow) rate of 100/sec., counts being added to the channel corresponding to that input voltage. Auxiliary circuitry was developed to convert the time-interval between successive impulses into a voltage proportional to that interval. Suitable

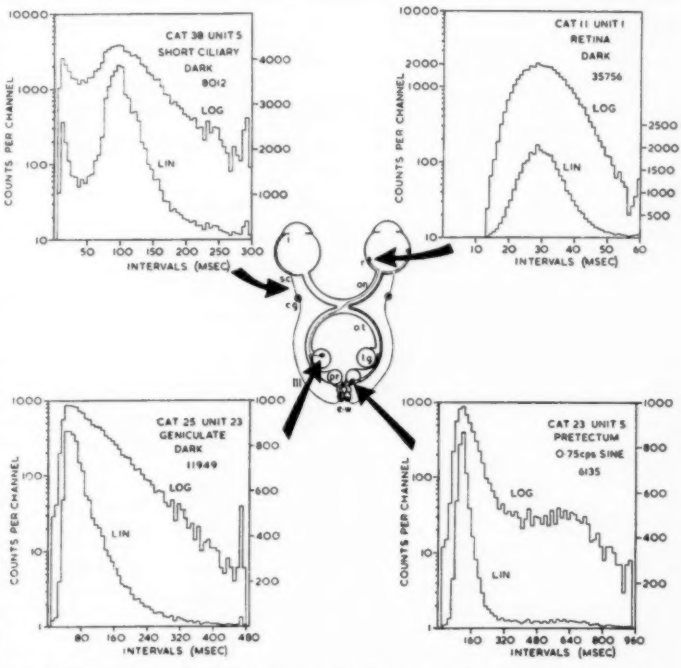


Fig. 1. Examples of interval histograms obtained from unit discharges recorded at four sites in the visual nervous system, as shown in the centre. The logarithmic scale of counts refers to the upper graph and the linear to the lower graph in each case

gating ensured that the analyser accepted not more than one sample of the voltage corresponding to any interval.

Certain limitations of this arrangement were recognized. Due to the fixed maximum sampling-rate of 100/sec., there was a probability that a sample would be missed whenever an interval in the discharge was 10 m.sec. or less. The particular sample missed would correspond to the interval preceding the short interval. If successive intervals are statistically independent, the missed samples will not affect the shape of the histogram obtained, but only scale down all channel counts by a constant factor, when sufficient intervals have been analysed to smooth out statistical fluctuations. However, since there was no prior knowledge that successive intervals in the nerve discharges were statistically independent, we have excluded those units in which missed samples amounted to more than 1 per cent of the total number of intervals examined.

The method has been successfully applied to unit activity recorded from four sites in the visual system of the cat: retina, lateral geniculate nucleus, pretectal nucleus and short ciliary nerve. Some examples of interval histograms obtained are shown in Fig. 1. Of immediate interest is the remarkable dissimilarity of the distributions from different regions. That of the retinal ganglion cell presented is of asymmetrical Gaussian type whereas the particular geniculate cell distribution seems best compared with a Poisson type. On the other hand, the short ciliary unit shows a bimodal distribution. It should be noted that counts due to intervals longer than the range of the analyser have been grouped into the last two channels.

Striking changes in the distribution of intervals occur when patterned photic stimulus is presented to the eye. This is demonstrated by the pretectal unit when the retina was stimulated by light, the intensity of which was modulated sinusoidally at 0·75 cycles/sec. frequency. There is a low, second mode in the distribution corresponding to a relative prevalence of long intervals corresponding to a silent period in the discharge during the cycle of light modulation.

This is a fast, flexible and accurate method. The analyses can be completed approximately 1,000 times more quickly than by conventional methods of photography and measurement of the enlargements. As can be seen from the graphs, a sixty-four-fold range of intervals can be examined at any particular setting of the analyser channel-width, and the channel-width can be set to any desired value to accommodate the discharge pattern of the particular unit. Large numbers of intervals can be examined (for example, 35,756 in the case of the retinal unit) for the total analyser count capacity is more than 8 million (2^{23}–64), and this is important in smoothing out the effects of statistical variation.

A large field has been made accessible and is being opened up by this technique. The information abstracted will ultimately form the foundation for the application of a communication theory to the visual system in particular and the nervous system in general.

This work was aided by grants from the National Health and Medical Research Council of Australia, the Ophthalmic Research Institute of Australia and the Post-Graduate Medical Foundation of the University of Sydney.

[1] Lampard, D. G., and Harvey, I. K., *J. Electronics and Control*, **9**, 233 (1960).
[2] Gerstein, G. L., *Science*, **131**, 1811 (1960).

HÆMIN-PROTEIN BINDING IN PEROXIDASE AND METHÆMALBUMIN

By Dr. A. C. MAEHLY*

Foundation for Medical Physics, University of Pennsylvania

WHEN protohæmin combines with the protein moieties of hæmoproteins its absorption maximum in the Soret region becomes intensified. This has been ascribed either to the establishment of an iron-protein bond or to a bond involving the propionyl side-chains of the hæmin.

O'Hagan and George succeeded in preparing artificial hæmoglobins[1] and myoglobins[2] with hæmins lacking carboxyl groups. They concluded that in these hæmoproteins the essential binding must involve the central iron atom. When coupling hæmin or hæm to human serum albumin, however, O'Hagan could demonstrate that the carboxyl groups of the hæmin molecule are involved in hæmin–protein binding[3]. Little and Neilands[4] have recently investigated the coupling of hæmins to human serum albumin.

Some simple experiments in this field were performed by me a few years ago. Since this work was interrupted, the experiments are reported in abridged form. Hæmin derivatives were prepared in which either the iron atom was missing (protoporphyrin) or the carboxyl groups were blocked (protohæmin-dimethyl ester). One or the other of these compounds was added to serum albumin or to apo-horseradish peroxidase, and the spectral changes in the Soret region recorded.

Human serum albumin was kindly supplied by Dr. M. Surgenor (then in Boston) in the form of Cohn's fraction V. Horseradish peroxidase was crystallized as described in ref. 5. Protoporphyrin IX-dimethylester was made according to Grinstein[6]. It melted at 221–225° uncorrected. Free protoporphyrin IX was obtained by careful hydrolysis of the ester with 25 per cent hydrochloric acid as described in ref. 7 and checking the absorption spectrum (peak at 412–413 mμ). Only freshly hydrolysed solutions were used. Protohæmin IX-dimethylester was made as in ref. 8. The crude product was purified on aragonite columns. This crystal form of

* Permanent address: Government Laboratory for Forensic Chemistry, Stockholm.

calcium carbonate proved to be superior to calcite or to mixtures of the two forms. (Thanks are due to Prof. F. Daniels (Madison, Wisconsin) for generous gifts of samples of aragonite and calcite.) The columns were prepared with dry benzene. A benzene solution of the crude protohæmin IX-dimethylester was added and the column developed with benzene. A green zone (*A*, about 5 per cent) travelled fastest; it was followed by a diffuse light-brown band (*B*, about 60 per cent) and a sharply defined dark brown zone (*C*, about 20 per cent). A brown area (*D*, about 12 per cent) stayed on top of the column. Benzene eluted *A* (not identified) then *B* (pure protohæmin IX-dimethylester), and benzene/chloroform (1 : 1) eluted *C* (the monomethyl ester ?). *D* proved to be free protohæmin, elutable by chloroform/acetic acid (99 : 1).

Human serum albumin binds only protoporphyrin dissolved in acid aqueous solutions. If the solutions are neutralized or made alkaline, no appreciable spectral change takes place on addition of human serum albumin, possibly due to polymerization. Fig. 1 shows the Soret spectra obtained on adding 40 μl. of a 128 μ*M* protoporphyrin solution in *N* hydrochloric acid to 2·20 ml. of 0·2 *M* Na_2HPO_4 on one hand, and to 2·20 ml. of 3·0 μ*M* human serum albumin in 0·2 *M* Na_2HPO_4 on the other hand. The final molar ratio protoporphyrin/human serum albumin was thus 2·4 : 1. Rosenfeld and Surgenor[9] had found that human serum albumin forms complexes with ferri protoporphyrin in the ratios of 1 : 1 and 1 : 2. When solutions of protoporphyrin-dimethylester or of protohæmin IX-dimethylester in acetone were added to buffered human serum albumin and to buffer alone respectively, no spectral differences between the two resultant solutions were obtained.

With horseradish peroxidase, similar, if somewhat less dramatic, results were obtained as with human serum albumin. Neither protoporphyrin-dimethylester nor protohæmin IX-dimethylester, both in acetone solution, was bound to apo-horseradish peroxidase. The spectra obtained on adding free protoporphyrin to apo-horseradish peroxidase are shown in Fig. 2. 40 μl. of a 79 μ*M* solution of protoporphyrin in 0·2 *N* hydrochloric acid were added to 2·20 ml. of 0·2 *M* phosphate buffer of *p*H 6·8, and to 2·20 ml. of 1·3 μ*M* apo-horseradish peroxidase in the same buffer respectively. The concentration of active apo-horseradish peroxidase was determined by spectrophotometric titration of the protein with protohæmin of known titre. The final concentration of protoporphyrin was thus 1·4 μ*M* and the molar ratios protoporphyrin/apo-horseradish peroxidase = 1·1 : 1·0. When the protoporphyrin concentration was chosen at a lower level, spectra intermediate between those shown in Fig. 2 were obtained; addition of larger amounts of protoporphyrin to both the blank and the 1 : 1 protoporphyrin–apo-horseradish peroxidase complex gave no further spectral change.

The maxima of the Soret bands for horseradish peroxidase, protoporphyrin–apo-horseradish peroxidase, methæmalbumin and protoporphyrin–albumin all lie at 403 mμ, irrespective of the presence or the absence of the central iron atom. Of more importance is the fact that the extinction coefficients of the porphyrin compounds are equal to or higher than those of the hæmin compounds. This is shown in Table 1.

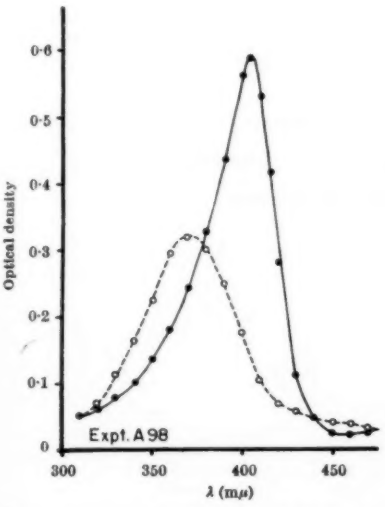


Fig. 1. Soret spectra of protoporphyrin IX in phosphate (Na_2HPO_4), in the absence (○ – ○) and in the presence (● – ●) of human serum albumin

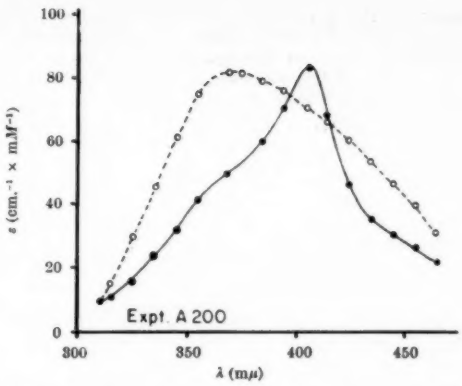


Fig. 2. Soret spectra of protoporphyrin IX in phosphate buffer, in the absence (○ – ○) and in the presence (● – ●) of apo-horseradish peroxidase

Table 1. Extinction Coefficients, $\epsilon_{m\mu}$, of Horseradish Peroxidase and Methæmalbumin and their Iron-free Counterparts at the Peak of the Soret Band at 403 mμ

Horseradish peroxidase	
recombined from apo-horseradish peroxidase and protohæmin, *p*H 6·8	81 cm.$^{-1}$mM^{-1}
Protoporphyrin–apo-horseradish peroxidase, *p*H 6·8	83 "
Methæmalbumin, *p*H 7–10 (cf. ref. 9)	81 "
Protoporphyrin human serum albumin, in Na_2HPO_4	116 "

A freshly prepared 1·3 μ*M* solution of the protoporphyrin–apo-horseradish peroxidase complex did not react with methyl hydrogen peroxide as judged by the appearance of the Soret spectrum. Protohæmin to a final concentration of 0·87 μ*M* was now added to blank and solution: no spectral change occurred. Renewed addition of methyl hydrogen peroxide (2 μ*M*) had no influence on the spectrum. When protohæmin was added directly to free apo-horseradish peroxidase, complex II was rapidly formed with a Soret peak at 418 mμ. The experiment shows that protoporphyrin competes with protohæmin for the same sites at the apo-horseradish peroxidase molecule. The results emphasize the im-

UNIVERSITY OF MICHIGAN LIBRARIES

XUM

portance of the propionyl carboxyl groups of porphyrins in binding with proteins. The intensification of the Soret band on binding seems to be mainly due to binding of the carboxyl group to the proteins investigated in the work described here.

[1] O'Hagan, J. E., *Biochem. J.*, **74**, 417 (1960).
[2] O'Hagan, J. E., and George, P., *Biochem. J.*, **74**, 424 (1960).
[3] O'Hagan, J. E., *Nature*, **184**, 1808 (1959).
[4] Little, H. N., and Neilands, J. B., *Nature*, **188**, 913 (1960).
[5] Maehly, A. C., *Methods in Enzymology*, **2**, 801 (Academic Press Pub., Inc., New York, 1955).
[6] Grinstein, M., *J. Biol. Chem.*, **167**, 515 (1947).
[7] Grinstein, M., and Wintrobe, M. M., *J. Biol. Chem.*, **172**, 459 (1948).
[8] Küster, W., and Schlayer, K., *Z. physiol. Chem.*, **168**, 302 (1927).
[9] Rosenfeld, M., and Surgenor, D. M., *J. Biol. Chem.*, **183**, 663 (1950).

TRANSFERRIN DIFFERENCES IN CHIMPANZEE SERA

By Prof. J. BUETTNER-JANUSCH

Laboratory of Physical Anthropology, Yale University

TRANSFERRIN exists in a variety of molecular species in man and other mammals[1]. Presumably the serum transferrins of the other Primates are similar to those of man. While screening blood of Primates for differences in hæmoglobin, transferrin, α_2-globulin and cæruloplasmin, we have been able to test a number of chimpanzee sera and find results both similar to and different from those reported by Boyer and Young[2].

The samples came from: four chimpanzees at the Department of Preventive Medicine at Yale; 19 sera, given to us by Dr. Edwin Isacson of Yale University, from animals at the Aero Space Medical Laboratory, Holloman Air Force Base, New Mexico; and 20 sera from the laboratory of Dr. Merris Goodman, Wayne State University, Detroit, Michigan. The specific identification of the animals is not certain, though we believe them to be *Pan troglodytes*. The variety of taxonomic labels given chimpanzees in the literature is not particularly informative. Most of the animals were reported to have come from Senegal, Sierra Leone and the French Cameroons. The 20 samples from Dr. Goodman came from Orange Park, Florida.

Sera were centrifuged at 16,000 r.p.m. at $-10°$ C.; 1·5 ml. of serum was treated for at least 1 hr. with 0·5 ml. of ferrous citrate containing iron-59, 10 μc./ml. The reaction was carried out either at ambient temperature (19° C.) or at 10° C. Serum samples were inserted directly into precast slots in 12–15 per cent starch–gels made with 0·021 *M* borate buffer, *p*H 8·6. The electrophoresis was run in the vertical position at 6 V./cm. for 16–18 hr. After the run was completed, each gel was sliced in half. One half was stained with amido-black dye in methanol/water/acetic acid solvent (5 : 5 : 1). The other half was wrapped in 'Saran Wrap' (Dow Chemical Corporation) and placed on a sheet of Kodak No-Screen X-ray film for 24 hr. The films were developed in Kodak *D*-8 or *D*-11 developer. The bands on the radiograph so produced were compared with various bands found on the section stained with amido-black. The serum patterns were compared in repeated runs with the serum patterns of Yale College students and with those of a number of other Primates.

A diagram of the transferrin types found is shown in Fig. 1. We cannot label our patterns with the same designations that Boyer and Young[2] gave their transferrin phenotypes, since we could compare their patterns with ours only on the basis of the position of band *C* from a human homozygous *TfC* individual. We have chosen to number our bands in order of decreasing mobility and name the types with inclusive numbers. We found eight separate, distinct transferrin bands on the various radiographs, though never more than five in one individual serum. We name the band with the greatest mobility number 1, the one with lowest, 8. For example, the serum which had five bands, 3, 4, 5, 6 and 7, we named type 3–7. In Fig. 1, human type *CC* is shown as well as one of the most frequent transferrins found in the African vervet or green monkey, *Cercopithecus aethiops*. Band 3, which corresponds to no other Primates' transferrin that we know, was usually the densest band on the radiographs. However, at least one of the eight bands showed up as a dense spot on the radiograph of at least one serum. Therefore, we see no reason for distinguishing them on the basis of the extent to which they affect the X-ray film. Table 1 shows the frequencies of the various types of transferrins.

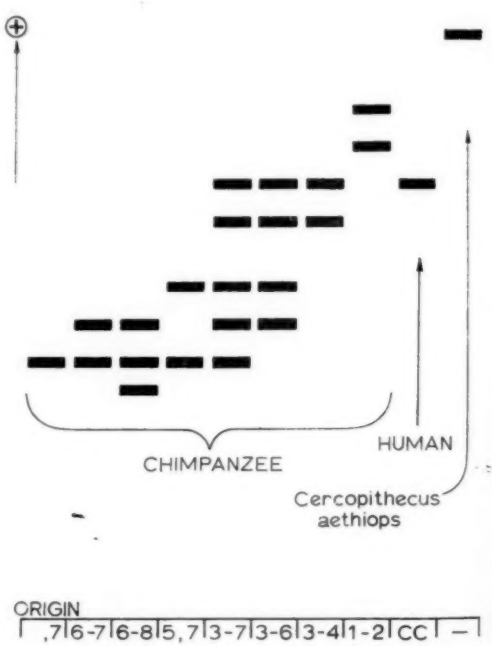


Fig. 1. Diagram of electrophoretogram illustrating relative mobilities of transferrin bands in various chimpanzees compared with a human *TfC* homozygote and a common transferrin type from *Cercopithecus aethiops*

Table 1. FREQUENCY OF TRANSFERRIN TYPES IN CHIMPANZEES

Type	Total	Number of animals* Group 1	Group 2	Group 3
1–2	1		1	
3–4	2		1	1
3–6 [*AC*]	2		2	
3–7 [*AD*]	7		7	
5, 7	2			2
6–7	14	1	2	11
6–8	12	2	4	6
,7	1		1	
	41†	3	18	20

* Group 1 are chimpanzees from Yale University; group 2 from Holloman Air Force Base; group 3 from Orange Park.
† Two sera of the original 43 produced unreadable patterns on each of three runs.

Exhaustive experiments in which each sample was saturated with iron-59 could not be undertaken because of the relatively small quantities of serum. Nevertheless, where we had enough material and could add a large excess of iron-59, we found that bands which were faint on the original radiograph continued to be faint after addition of an excess of iron-59. The possibility occurs that the degree to which the transferrins are saturated *in vivo* before the blood samples were taken plays a part in the extent to which each band will take up iron-59 *in vitro* (Bearn, A., private communication).

It seems reasonable to identify our 3–7 type with the *AD* type of Boyer and Young, and our 3–6 type with their *AC*. The other types do not correspond. The lack of correspondence may be due to species differences. Our chimpanzees belong to a group labelled *Pan troglodytes*; *Pan satyrus*, the name given the chimpanzees from which Boyer and Young received samples, is not known to us and is not recognized as a specific name by Fiedler[3], the name list at present in widest use. It is possible that these are the dwarf varieties of the Congo chimpanzee described by Schwarz[4]. Boyer (private communication) has stated recently that his animals were identified as *Pan troglodytes* by W. L. Straus, jun.

Another possibility is that these results are an additional demonstration of the marked polymorphism that chimpanzees show for the transferrin trait. Most of the animals showed the 6–8 and 6–7 patterns. The other sera were distributed more or less randomly among the other types. In other words, there are no marked frequency differences between the Holloman chimpanzees and the Orange Park chimpanzees, despite apparent differences in place of origin in Africa.

The serum patterns of these chimpanzees were compared with all the other Primates available to us. One of our most striking findings is that only *Tupaia glis*, of all the Primates tested, showed iron-binding β-globulins which moved to the same general area on the gels as did those of the chimpanzee. All other Primates available to us, except man (we have not been fortunate enough to obtain a sample from *Tarsius* yet), showed iron-binding bands of the β-globulins which have two and even three times the mobility of those of the chimpanzee.

We found early in our work that it is best, as Boyer and Young showed, to use borate buffers in the gels and in the electrode trays in order to obtain the fine resolution of chimpanzee transferrin bands necessary to demonstrate multiple zones. No other Primates, however, showed more than three iron-binding zones, though many other bands appear in the β-globulin region when macaque sera are subjected to electrophoresis in vertical starch-gels[5].

Unfortunately, it is not possible, at present, to discuss the possible modes of inheritance of this trait in the chimpanzee. There are no good pedigrees available now, and it is unlikely that matings critical for working out the inheritance will occur.

The multiplicity of the transferrin bands is similar to the situation in cattle β-globulins reported by Ashton[6]. It is not clear yet whether all the bands in the β-globulin region of Primates represent separate alleles or whether the mode of inheritance is more complex. The situation is made more complicated by the fact that the transferrin bands and the β-globulins do not show a one-to-one correspondence. A more detailed account of the β-globulins in Primates will be published later.

I wish to thank Dr. Edwin Isacson and Dr. Francis Black of Yale University and Dr. Morris Goodman of Wayne State University for their co-operation. The work on this project was performed under grant *RG*-6053 of the United States Public Health Service.

[1] Smithies, O., and Hiller, O., *Biochem. J.*, **72**, 121 (1959).
[2] Boyer, S. H., and Young, W. J., *Nature*, **187**, 1035 (1960).
[3] Fiedler, W., *Primatologia*, **1**, 1 (1956).
[4] Schwarz, E., *Rev. Zool. Bot. Afr.*, **16**, 425 (1929).
[5] Kirk, R. L., and Lal, L. Y. C., *Nature*, **188**, 673 (1960).
[6] Ashton, G. C., *Nature*, **180**, 917 (1957); **182**, 370 (1959); **184**, 1135 (1960).

MECHANISM OF STREPTOMYCIN ACTION ON BACTERIA: A UNITARY HYPOTHESIS

By DR. CHARLES R. SPOTTS* and PROF. R. Y. STANIER
Department of Bacteriology, University of California, Berkeley

THE response of a bacterium to the presence of streptomycin in its environment is genetically determined, and can take three alternate forms, commonly known as sensitivity, resistance and dependence. For a sensitive strain, streptomycin is toxic at very low concentrations; for a resistant strain, it is without effect on growth at considerably higher concentrations; and for a dependent strain, it is an essential growth factor. Several different states are included under the broad heading of resistance, only one of which will be dealt with here. We shall be concerned with the state in which a high level of resistance is acquired as a result of a single-step mutation from sensitivity, a type of resistance that is called indifference by some authors.

In *Escherichia coli*, genetic analysis has shown that sensitivity, dependence and single-step, high-level resistance are determined by multiple alleles of a single genetic locus[1–4]. In terms of modern genetic theory, this fact implies that the three phenotypes are ultimately determined by alternate states of a specific protein, or of a regulatory system that con-

*Present address: Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, Gif-sur-Yvette (Seine-et-Oise), France.

UNIVERSITY OF MICHIGAN LIBRARIES
XUM

trols the synthesis of a specific protein. Hence all the epiphenomena that are specifically associated with each of these three phenotypes should fall into a causal chain, and the three causal chains should all lead back ultimately to three alternate functional states of the streptomycin locus. This fundamental logical inference from the genetic data has been ignored in most theorizing about the mode of action of streptomycin. Instead, there has been a regrettable tendency to embellish the account of each newly discovered effect of streptomycin on bacteria with yet another topical 'explanation' of the action of streptomycin.

Most work on the physiological and biochemical effects of streptomycin on bacteria has been conducted with sensitive and resistant strains. This preoccupation with sensitivity and resistance is mirrored by present explanatory hypotheses, which are directed primarily or exclusively towards interpreting the epiphenomena associated with these two states, and do not attempt to account for the epiphenomena associated with dependence. One of us (C. R. S.) has recently made a detailed physiological and biochemical study of streptomycin dependence in *Escherichia coli*. The hypothesis which we shall present here is based primarily on the resultant findings; but it does have the somewhat novel advantage of explaining, in terms of structural modifications at a single intracellular site, some of the specific epiphenomena associated with sensitivity and resistance.

Epiphenomena associated with Dependence on Streptomycin

Full experimental details of our work on streptomycin dependence will be published elsewhere[5]. For the present purposes, it is sufficient to describe in summary fashion the rather complex epiphenomena which have been revealed by this work and by the work of earlier investigators.

(1) A relatively high external concentration of streptomycin (greater than 100 μgm./ml.) is necessary for growth of a dependent strain at maximal rate; when the streptomycin concentration falls below this *critical level*, the growth-rate becomes dependent on, and proportional to, streptomycin concentration[3–5]. For any given strain, the absolute magnitude of the critical level varies in response to such factors as the salt content of the medium, the nature of the carbon source and the temperature. At very low streptomycin-levels exponential growth can no longer be maintained; growth becomes arithmetic.

(2) When the streptomycin-level of the medium is above the critical one, growing cells of a dependent strain have what we shall define as a normal macromolecular composition. Under these conditions, the cellular content of protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) does not differ significantly from that of the corresponding sensitive strain, growing under identical environmental conditions in the absence of streptomycin[5]. However, when the streptomycin concentration of the medium is below the critical level, the macromolecular composition of the dependent cells becomes abnormal; and the degree of the abnormality is systematically related to streptomycin concentration[5]. At progressively lower streptomycin-levels, the RNA content of the cells becomes greater, and the protein content becomes smaller. DNA content is slightly sub-normal at low streptomycin-levels. The effects of streptomycin concentration on cellular composition are shown in Fig. 1.

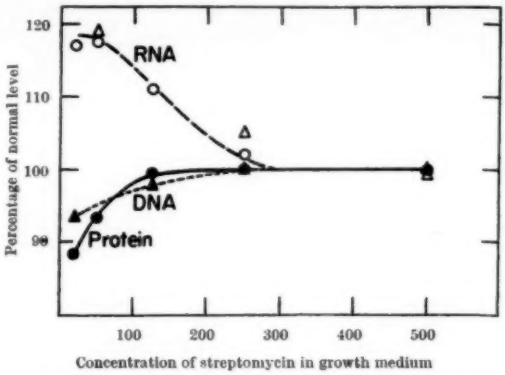


Fig. 1. Effect of the streptomycin concentration of the medium on cellular composition of a dependent strain of *Escherichia coli*

(3) If dependent cells which have been grown with a supra-critical concentration of streptomycin are washed and re-suspended in a streptomycin-free medium, they continue to divide for a limited period; the number of divisions is a function of the concentration of streptomycin to which they were previously exposed[1,9]. After the cessation of cell division, the cells can elongate[7,9,10], and further nuclear divisions can occur[11]. A systematic analysis of this residual growth has revealed other significant facts[5]. The cell mass increases exponentially at maximal rate for a short period, the duration of which is directly proportional to the streptomycin concentration of the medium from which the cells were taken. The growth-rate then declines progressively over a period of 1–2 hr., after which growth becomes arithmetic. Arithmetic growth, accompanied by an approximately six-fold increase of cell mass, continues for 16–20 hr. before the stationary phase is finally attained. The cessation of cell division coincides roughly with the termination of exponential growth; consequently the increase of cell mass during arithmetic growth is accompanied by a progressive elongation of the cells.

(4) Deprivation of streptomycin is not immediately lethal: the size of the viable population, fixed by the cessation of cell division, does not begin to decline until several hours after attainment of the stationary phase[5].

(5) The initial period of residual growth is accompanied by a continuous and systematic change in the macromolecular composition of the cells[5]. In effect, such cells pass through the series of fixed compositional states which occurs in cultures that are grown at different sub-critical levels of streptomycin. When the exponential phase of residual growth terminates, the cells have reached a state of extreme compositional imbalance (high RNA content, low protein and DNA contents) which is thereafter maintained throughout the course of arithmetic growth[5].

(6) If a supra-critical level of streptomycin is produced in a population in the course of arithmetical deprived growth, DNA synthesis rapidly regains its normal exponential rate. The rate of protein synthesis also increases very shortly after streptomycin is added, but attains the normal exponential level a little more slowly. The net synthesis of RNA ceases completely until the RNA-level of the cells has fallen by dilution to its normal value, after which RNA synthesis is resumed at the normal exponential rate[5].

Table 1. Effects of Streptomycin Deprivation on Enzyme Synthesis by a Dependent Strain of *Escherichia coli*

Functional class	Intracellular location	Enzyme	Specific activity, per cent of normal*
Respiratory	Soluble	Isocitric dehydrogenase	60
	Particulate	DPNH oxidase	60
	Particulate	Succinic dehydrogenase	40
	Gross respiratory activity (Q_{O_2})		45–55
Biosynthetic	Soluble	Threonine deaminase	100
	Soluble	5-Dehydroshikimic reductase	100
	Soluble	Glutamic dehydrogenase	50
	Soluble	Tryptophan synthetase	20
	Particulate	Dihydroorotic dehydrogenase	60
Non-functional under the conditions of cultivation used†	Soluble	Glucose-6-phosphate dehydrogenase	95
	Soluble	β-Galactosidase	5

*The specific activities (on a protein basis) of the enzymes examined remain constant throughout the arithmetic phase of deprived growth, and these values are taken to represent the deprivation-levels. The 'normal' specific activity is that found in the cells during their immediately preceding exponential growth in a medium of the same composition, supplied with a supra-critical level of streptomycin.

†The strain utilized for these experiments is constitutive for the production of β-galactosidase. The medium contained glycerol as the sole source of carbon and energy.

(7) Generally speaking, the intracellular state of the RNA in dependent cells growing with a critical level of streptomycin is 'normal'; that is, it cannot be distinguished from that in sensitive cells of the same species growing under similar conditions without streptomycin. The quantitative distributions of RNA between soluble and ribosomal fractions are closely similar; the sedimentation patterns of the ribosomes are identical; and the isolated ribosomes contain the same amounts of protein and RNA. Streptomycin deprivation does not affect the intracellular state of RNA in dependent cells in any of the respects already mentioned. Consequently, the increase in the total RNA content of the cells during deprivation simply reflects a very high differential rate of synthesis of *all* forms of cellular RNA[5].

(8) Since deprived cells have an abnormally high content of ribosomes, they also have an abnormally high content of ribosomal protein. Hence the cellular content of non-ribosomal protein—broadly speaking, the total complement of enzymes—must fall very substantially as a result of deprivation. Analyses of the enzymatic constitution of cells undergoing deprivation show that the decline in level is not uniformly shared by all the enzymes of the cell[5]. The synthesis of some is virtually abolished; the synthesis of others is maintained at a rate which is in some cases normal and in some cases reduced, relative to the rate of total protein synthesis. A summary of these data (Table 1) shows that the selective effects of deprivation on enzyme synthesis are essentially random. No particular class of enzymes is specifically affected; nor is there a specific conservation of those enzymes essential for the maintenance of physiological function.

(9) The streptomycin-dependent strain of *E. coli* used in our experiments was lysogenic for phage λ, the formation of which can be induced by ultra-violet irradiation. Even under conditions of severe streptomycin deprivation (that is, during the arithmetic phase of deprived growth) ultra-violet irradiation of the cells causes induction, with the subsequent lysis of the cells and liberation of active phage particles[5]. Hence streptomycin deprivation does not prevent the synthesis by the dependent cell of functional DNA.

(10) During the growth of a dependent strain, no change in the streptomycin content of the medium can be detected[5,12]. A previous attempt to show streptomycin uptake by the cells[13] gave negative results. However, by the use of radioactive streptomycin, we have been able to show that dependent cells bind a small amount[5]. This bound streptomycin cannot be removed from the cells by repeated washings with phosphate buffer or with non-radioactive streptomycin. The cells were grown in a medium that contained radioactive streptomycin at a concentration which was about double the critical level. The amount of radioactivity in the cells was very close to the limits of detection, but with due regard for a large possible experimental error, we can say that under these conditions each cell binds irreversibly some 250,000 molecules of streptomycin. Approximately half is extractable by hot water; the remainder is unextractable with cold perchloric acid, but can be completely extracted with hot perchloric acid.

Interpretations of the Epiphenomena associated with Dependence

We assume that the dependent cell contains certain specific receptor sites, all of which must be combined with streptomycin for the maintenance of normal cellular function. We further postulate that there is a relatively large number of such sites, all of which are identical in structure and in combining power for streptomycin. When the medium has a critical streptomycin-level, the streptomycin concentration within the cells is just sufficient to keep all the receptor sites of the growing population combined with streptomycin. At sub-critical levels, the internal streptomycin concentration is insufficient to keep all receptor sites combined, and cellular function becomes deranged. At supra-critical levels, the streptomycin within the cells is more than sufficient to keep all receptor sites combined, and there is consequently an uncombined pool, the size of which is related to the external concentration. This pool is probably loosely held by other cellular constituents, since it cannot exchange with external streptomycin, but can be extracted with hot water.

We suggest that the intracellular receptor sites are the ribosomes, and that the firmly bound streptomycin in the cell (that is, the fraction unextractable by hot water) is all attached to ribosomes. The number of molecules of streptomycin so bound by each cell is of the order of 100,000, so that there would be relatively few molecules (perhaps only one) bound to each ribosome. Let us now see how these assumptions can be used to account for the distinctive epiphenomena which accompany streptomycin deprivation: namely, the selective repression of the synthesis of some enzymes, and the over-production of all forms of cellular RNA.

According to present concepts about the mechanism of protein synthesis, the ribosomes act as non-specific assembly sites for the manufacture of proteins[14–16]. The specific protein which is synthesized

UNIVERSITY OF MICHIGAN LIBRARIES

XUM

at any given time on any given ribosome is determined by the transient attachment to the ribosome of a short-lived 'messenger RNA'[17-21]. The various messenger RNA's are direct gene products, each of which carries in its unique base sequence a copy of the genetic code. This base sequence is presumed to specify the amino-acid sequence of the protein molecule that is assembled on the ribosome.

We propose that mutation to dependence at the streptomycin locus causes a slight modification in the structure of all ribosomes. Although this modification is insufficient to change the gross properties of the ribosomes (as evidence by their normal sedimentation pattern and gross composition), it makes them unable to combine as effectively as ribosomes of the sensitive phenotype with certain messenger RNA's. The defect in combining power can be repaired by the attachment of streptomycin to the ribosome, an event which we shall call activation. The ribosomal structure characteristic of a dependent cell can have three possible effects on the synthesis of specific proteins.

(*A*) If a specific messenger RNA has no affinity for the ribosome of the dependent phenotype, ribosomal activation with streptomycin is indispensable for synthesis of the corresponding protein.

(*B*) If a specific messenger RNA has a reduced affinity for the ribosome of the dependent phenotype, activation with streptomycin is necessary for synthesis of the corresponding protein at maximal rate.

(*C*) If a specific messenger RNA has an unchanged affinity for the ribosome of the dependent phenotype, the rate of synthesis of the corresponding protein is not increased by activation with streptomycin.

After growth with a supra-critical concentration of streptomycin, dependent cells have a fully activated complement of ribosomes, and in addition contain an internal pool of streptomycin, proportional in size to the external concentration. In the absence of external streptomycin, such cells can continue normal growth as long as newly formed ribosomes can be fully activated at the expense of the internal pool. As soon as the pool is exhausted, the rates of synthesis of proteins belonging to categories *A* and *B* begin to fall. This event is translated into exponential growth at ever-diminishing rate, characteristic of the first period of deprivation. The concentration of activated ribosomes in the cells eventually falls to a point at which proteins belonging to category *A* practically cease to be formed, since the probability of the corresponding messenger RNA finding an activated ribosome during its short lifetime has become small. Provided that some of these proteins play an essential catalytic part, growth then becomes arithmetic, as actually observed in the second period of deprivation.

We must now see what interpretation can be offered for the increase in the rate of RNA synthesis which sets in as soon as the streptomycin-level begins to limit the growth-rate. Magasanik and Neidhardt[22,23] have made a detailed study of the control of RNA and protein synthesis during normal bacterial growth. Their findings suggest that growth-rate (which is essentially a reflexion of the rate of total protein synthesis) is determined by the concentration of RNA in the cell. This implies the existence of a feed-back control on RNA synthesis. When a cell is placed under conditions where biosynthetic precursors can be manufactured more rapidly than they can be used for protein synthesis, a larger fraction of the flow of carbon and nitrogen is directed into the synthesis of RNA and of ribosomal protein. This preferential synthesis continues until the limitation placed on the rate of protein synthesis has been overcome, and the cell has achieved metabolic balance under a new set of steady-state conditions.

The growth of dependent cells with sub-critical levels of streptomycin provides the first known instance of an *inverse* relationship between growth-rate and cellular RNA content. How is this apparent anomaly to be explained? As soon as the streptomycin concentration in a dependent cell falls to a level that is no longer sufficient to activate all ribosomes, the rate of protein synthesis begins to fall and biosynthetic precursors begin to accumulate. At this point, the feed-back control mechanism comes into play, and a larger flow of raw materials is directed into the formation of RNA and ribosomal protein. But in the dependent cell under the conditions of streptomycin deprivation, the production of more ribosomes only accentuates the imbalance which it would correct in a normal cell, since it decreases the rate of synthesis of all proteins belonging to categories *A* and *B*. The feed-back control mechanism goes out of regulation, and more and more ribosomes and soluble RNA are made by the cell. The ribosomes can, it is true, serve for the synthesis of proteins belonging to category *C*; but since the proteins of this category constitute an arbitrary array, in terms of their physiological function, the only consequence is to establish a condition of enzymatic imbalance. This affects not only biosynthetic systems, but also the systems responsible for energy-yielding metabolism, as shown by the fact that deprivation can result in a substantial depression of the Q_{O_2} of the cells[5,24].

The ribosomes made during deprivation are potentially fully functional; and the addition of streptomycin to the deprived culture causes their rapid activation. As a consequence, the rate of synthesis of the proteins belonging to categories *A* and *B* immediately rises relative to that of proteins belonging to category *C*. As the enzymatic imbalance in the cells is overcome, exponential growth can be resumed. With the rise in the rate of protein synthesis that follows the re-introduction of streptomycin, the feed-back control on RNA synthesis again comes into normal operation, with the consequence that further net synthesis of RNA is totally repressed until the ribosomal content of the cells has been reduced by growth to a level that once again limits protein synthesis.

The hypothesis which has been outlined here is admittedly complex, and constructed on indirect experimental evidence. Nevertheless, it provides a logically coherent formal explanation for *all* the distinctive epiphenomena associated with streptomycin dependence. It also leads to many explicit predictions, some of which should be susceptible of immediate experimental test.

As emphasized in the introduction, any hypothesis concerning the mode of streptomycin action on bacteria must be deemed inadequate unless it can explain in functional terms the allelism of sensitivity, resistance and dependence. In the remaining section of this essay, we shall accordingly show briefly how this hypothesis can be extended to interpret sensitivity and resistance.

Interpretation of Sensitivity and Resistance

We have postulated that in the dependent phenotype the ribosomes have undergone a slight structural

modification which reduces their affinity to a greater or less extent for some messenger RNA's ; and further, that a normal affinity can be restored by activation with streptomycin. We are therefore led to assume that the sensitive and resistant phenotypes are, in the last analysis, expressions of other structural states of the ribosomes. In both these phenotypes, it is obvious that the ribosomes have full affinity for all messenger RNA's in the absence of streptomycin. Hence the postulated structural difference between them is of such a nature that its functional consequences are expressed only in the presence of streptomycin. We propose that the ribosomes of sensitive cells have a structure that confers on them a very high affinity for streptomycin, and that the resultant combination with streptomycin wholly prevents the attachment of messenger RNA's, thus abolishing protein synthesis. The ribosomes of resistant cells, on the other hand, have a structure such that they cannot combine with streptomycin ; hence streptomycin cannot impede the attachment of messenger RNA's, and the synthesis of proteins.

The first obvious prediction which can be made on the basis of this suggestion is that sensitive and resistant cells should have markedly different binding capacities for streptomycin. This is confirmed by the recent work of Hancock[25]. Placed in the presence of a minimal inhibitory concentration of streptomycin, sensitive cells can bind a small quantity irreversibly. The quantity so bound is of the same order as that which we find to be bound by dependent cells placed in the presence of a far higher external streptomycin concentration. Resistant cells, on the other hand, bind a relatively insignificant amount of streptomycin[25]. The much lower binding capacity of the resistant cell could, of course, be explained by the assumption that streptomycin cannot enter cells of this phenotype. However, if a permeability change is invoked to explain resistance, its allelism with dependence and sensitivity should imply that these two phenotypes are also basically determined by special states of the permeability barrier (presumably the cell membrane). At least for dependence, such a premise is wholly devoid of explanatory value.

Another prediction which follows from our hypothesis is that streptomycin should act as a very powerful inhibitor of protein synthesis in cells of the sensitive phenotype, but not in cells of the resistant phenotype. This prediction is confirmed by the observations of Erdös and Ullman[26,27]. They have found that in extracts of cells of the sensitive phenotype, low concentrations of streptomycin abolish amino-acid transfer from soluble RNA to protein ; this effect of streptomycin is not demonstrable in extracts of cells of the resistant phenotype. The demonstration of this differential effect of streptomycin on protein synthesis in cell-free systems is of particular importance, since it reflects a major phenotypic difference between the sensitive and the resistant cell which cannot be attributed to a difference in permeability.

One of us (C. R. S.) thanks the National Institutes of Health, U.S. Public Health Service, for a predoctoral fellowship, during the tenure of which this work was carried out.

Note added in proof. Dr. D. T. Dubin and Prof. B. D. Davis have recently observed that exposure of a sensitive strain of *E. coli* to streptomycin results in the intracellular breakdown of the ribosomes (personal communication). This fact is fully consonant with our general hypothesis concerning the primary site of streptomycin action in bacteria, but implies that the special interpretation of sensitivity which we present must be amended: streptomycin does not simply inactivate the ribosomes of the sensitive phenotype; it destroys them. Indeed, this amendment makes possible a much more satisfactory explanation of the various epiphenomena associated with sensitivity than does our original interpretation.

[1] Demerec, M., Wallace, B., Witkin, E. M., and Bertani, G., *Carnegie Inst. Wash. Yearbook*, **48**, 154 (1949).
[2] Newcombe, H. B., and Nyholm, M. H., *Genetics*, **35**, 603 (1950).
[3] Lennox, E. S., *Virology*, **1**, 190 (1955).
[4] Hashimoto, K., *Genetics*, **45**, 49 (1960).
[5] Spotts, C. R. (in preparation).
[6] Paine, jun., T. F., and Finland, F., *J. Bacteriol.*, **56**, 207 (1948).
[7] Schaeffer, P., *Ann. Inst., Pasteur*, **78**, 634 (1950).
[8] Sulitzeanu, D., and Olitzki, A. L., *Exp. Med. Surg.*, **13**, 394 (1955).
[9] Bertani, G., *Genetics.*, **36**, 598 (1951).
[10] Simon, E., Ph.D. Thesis, University of Wisconsin (1955).
[11] Delaporte, B., *Carnegie Inst. Wash. Yearbook*, **48**, 166 (1949).
[12] Rubin, B. A., and Steinglass, P., *Bacteriol. Proc.*, p. 33 (1951).
[13] Szybalski, W., and Mashima, S., *Biochem. Biophys. Res. Comm.*, **1**, 249 (1959).
[14] McQuillen, K., Roberts, R. B., and Britten, R. J., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1437 (1959).
[15] Cohen, G. N., and Gros, F., *Ann. Rev. Biochem.*, **29**, 525 (1960).
[16] Novelli, G. D., *Ann. Rev. Microbiol.*, **14**, 65 (1960).
[17] Volkin, E., and Astrachan, L., *Virology*, **2**, 149 (1956).
[18] Nomura, M., Hall, B. D., and Spiegelman, S., *J. Mol. Biol.*, **2**, 306 (1960).
[19] Brenner, S., Jacob., F., and Meselson, N., *Nature*, **190**, 576 (1961).
[20] Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Risebrough, R. W., and Watson, J. D., *Nature*, **190**, 581 (1961).
[21] Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).
[22] Magasanik, B., Magasanik, A. K., and Neidhardt, F. C., in *The Regulation of Cell Metabolism* (Ciba Foundation Symposium ; Little, Brown and Co., Boston, 1959).
[23] Neidhardt, F. C., and Magasanik, B., *Biochim. Biophys. Acta*, **42**, 99 (1960).
[24] Schaeffer, P., *C.R. Acad. Sci., Paris*, **228**, 440 (1949).
[25] Hancock, R., *Biochem. J.*, **78**, 7P (1961).
[26] Erdös, T., and Ullmann, A., *Nature*, **183**, 618 (1959).
[27] Erdös, T., and Ullmann, A., *Nature*, **185**, 100 (1960).

TEMPERATURE CHARACTERISTICS OF BACTERIAL VARIATION IN LEGUME SYMBIOSIS

By Dr. J. S. PATE

Department of Botany, Queen's University, Belfast

THERE is abundant evidence of wide variation within the legume nodule organism *Rhizobium*. Cultural[1] and serological[2] characters have been used to define the free-living bacteria, while their behaviour in association with various legumes has been shown to range from non-invasibility[3] or infection but inability to nodulate[4] to all grades of harmony in symbiosis[5–7].

It is usual to describe bacterial variation in symbiosis by comparing the nodulating and nitrogen-fixing properties of various bacterial partnerships of one host genotype under identical growth condi-

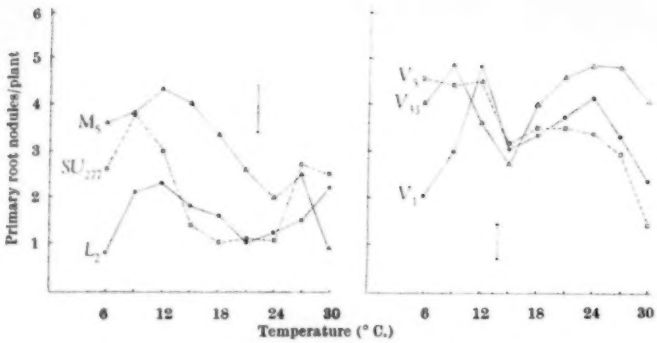


Fig. 1. Effect of a range of constantly maintained temperatures on the nodulating performances of selected strains of nodule bacteria on barrel medick (*Medicago tribuloides* Desr.) on the left, and purple vetch (*Vicia atropurpurea* Desr.) on the right. Code numbers refer to strains of *Rhizobium*. Plants grown at 400 ft.-candles light intensity, 99 per cent confidence limits are included

tions. The purpose of this article is to report a new type of symbiotic variation where reaction to a range of environmental conditions is used to determine the physiological characteristics of host plant and invading bacterium.

Purple vetch (*Vicia atropurpurea* Desf.) and barrel medick (*Medicago tribuloides* Desr.) were paired with selected strains of nodule bacteria of Australian or British origin. Each host–bacterium combination was grown in culture rooms maintained at a series of constant temperatures (6–30° C.). The nitrogen-free sand culture technique was used as described previously[8]. Each growth series received 16 hr./day of fluorescent lighting giving intensities of 400, 600, or 800 ft.-candles at sand-level in the culture vessels.

In work on nodulation response to temperature, the collection of the plants was timed to coincide with the completion of nodulation of the seedling primary root. Average nodule number in the 50-plant sample from the 5 replicate pots of a treatment was determined as a measure of initial response to infection. Clearing roots in chloral hydrate facilitated the counting of the small nodules developed by some bacterial strains in unfavourable environments. Fig. 1 illustrates typical nodulation performances in vetch and medick. All nodulation curves show optima at or near the extremes of growth temperatures, and comparisons of the relative magnitudes of these pairs of optima would indicate that certain bacteria (for example, V_{33} and L_2) are particularly effective in forming nodules at high temperatures. Other bacterial strains (M_5 and V_1) illustrate the opposite effect. Several investigations[9–10] have suggested that an existing nodule may exert inhibitory influences on its younger companions, and such a hypothesis might interpret the general depression of nodulation in the middle of the temperature-range as a direct consequence of a more active production of inhibitory substances by the first-formed nodules. Similarly, a specific bacterial contribution to the production of inhibitory substances might account for the different temperature characteristics recorded in the nodulation of each partnership of a host plant.

Results of a preliminary experiment on the interaction of light and temperature on the nitrogen fixation of bacterial associations of vetch and medick are shown in Fig. 2. The values quoted refer to the first 5 weeks of nitrogen fixation, commencing when nodules first acquired visible hæmoglobin pigmentation. In both species nitrogen fixation shows a well-defined maximum at 24° C., with symbiosis in barrel medick clearly more tolerant of high temperatures than in purple vetch (compare nodule formation, Fig. 1). The higher light intensity benefits fixation particularly at low or medium temperatures. The two barrel medick associations offer evidence of a bacterial factor influencing variations in symbiotic activity over the temperature-range. Thus the L_2 strain shows significantly greater fixation at lower temperatures, while the reverse obtains at temperatures at or above 24° C. These differences are reproduced in both light conditions and, like the initial infection responses, appear to represent minor variations on the general pattern of host-response to temperature.

Table 1 records nitrogen analyses of dry weights for typical bacterial associations of vetch and medick harvested after 5 weeks of nitrogen fixation at 600 ft.-candle light intensity.

A specific inhibition of fixation processes by growth temperatures at or above 27° C. is suggested in both associations from the low values for percentage nitrogen in dry weight. By contrast, the depression of total fixation at or below 21° C. (see Fig. 2) is seen to be associated with the higher values for nitrogen percentage, suggesting that growth processes other than nitrogen fixation are retarded in conditions of lower temperatures.

The two species studied here may be grouped with those described by Mes[11] (*Vicia sativa*, *Pisum sativum*,

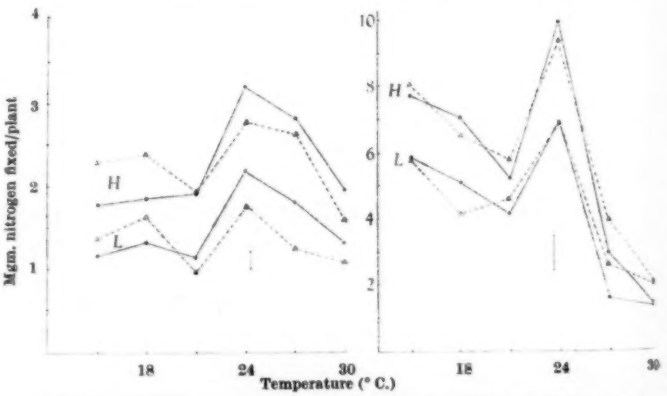


Fig. 2. The effects of a range of constantly maintained temperatures and two light intensities on nitrogen fixation by bacterial associations of medick (left) and vetch (right). Code numbers refer to strains of *Rhizobium*. 99 per cent confidence limits are derived from the 50-plant sample (5 replicate pots) of each treatment. *H*, 800 ft.-candles; *L*, 400 ft.-candles light intensity; for medick, ●, BM_{101}; ▲, L_2; △ for vetch, ●, V_{33}; △, V_1

Table 1. Dry Weight Analysis of Nodulated Vetch and Medick Plants grown in Nitrogen-free Culture at a Range of Constant Temperatures

Growth temp. (° C.)		15	18	21	24	27	30
Nitrogen fixed as a % of plant dry weight*	Purple vetch—*Rhizobium* V_1	4·4	4·7	4·8	4·1	3·8	2·9
	Barrel medick—*Rhizobium* BM_{104}	3·9	4·3	4·4	3·9	3·7	3·0

* Corrected to exclude nitrogen furnished by cotyledons

Lupinus luteus) and Meyer and Anderson[12] (*Trifolium subterraneum*) as showing a specific suppression of symbiotic fixation at high growth temperatures. Mes[11,13] has also described the opposite condition, where nitrogen fixation is specifically depressed by low temperatures (for example, in *Arachis hypogaea*, *Stizolobium deeringianum*, and *Glycine max*). The present work on *Medicago tribuloides* would suggest the possibility of selecting bacterial strains to combat such limitations of symbiosis in unfavourable growth conditions.

Further experiments are being conducted to investigate symbiotic activity where host plants are subjected to a range of further environmental factors, including seasonal and diurnal thermo-periodicity and the presence of varied amounts of combined nitrogen in the rooting medium.

[1] Fred, E. B., Baldwin, I. L., and McCoy, E., *Root Nodule Bacteria and Leguminous Plants* (University of Wisconsin Press, 1932).
[2] Vincent, J. M., *Proc. Linn. Soc. N.S.W.*, **66**, 145 (1941).
[3] Allen, O. N., and Baldwin, I. L., *Soil Sci.*, **78**, 415 (1954).
[4] Allen, E. K., and Allen, O. N., *Bact. Rev.*, **14**, 273 (1950).
[5] Virtanen, A. I., *Ann. Rev. Microbiol.*, **2**, 485 (1948).
[6] Nutman, P. S., *Biol. Rev.*, **31**, 109 (1956).
[7] Allen, E. K., and Allen, O. N., in *Encyclopedia of Plant Physiology*, **8**, 48 (Springer-Verlag, Berlin, 1958).
[8] Dart, P. J., and Pate, J. S., *Austral. J. Biol. Sci.*, **12**, 427 (1959).
[9] Nutman, P. S., *Ann. Bot.*, **13**, 261 (1949).
[10] Nutman, P. S., *Ann. Bot.*, **16**, 80 (1952).
[11] Mes, M. G., *Nature*, **183**, 2032 (1959).
[12] Meyer, D. R., and Anderson, A. J., *Nature*, **183**, 61 (1959).
[13] Mes, M. G., *S. Afric. J. Sci.*, **55**, 35 (1959).

CRYSTALLIZATION OF CYTOCHROME b_2 FREE OF THE DEOXYRIBONUCLEIC ACID COMPONENT

By Prof. R. K. MORTON and KATHRYN SHEPLEY

Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide

CRYSTALLINE cytochrome b_2 was isolated in 1954 by Appleby and Morton[1,2], who showed that it was identical with L(+)-lactate dehydrogenase of baker's yeast. The crystalline enzyme is a flavohæmoprotein[1,3,4]. It contains about 5 per cent of a deoxyribonucleic acid (DNA)[5,6] which appears to be homogeneous and which has a molecular weight of 10,000–12,000 as estimated by end-group analysis[7]. The DNA-component may be dissociated from the flavohæmoprotein by dialysis against a 70 per cent saturated solution of ammonium sulphate[6,7] without any substantial change in the specific dehydrogenase activity of the enzyme[2,6]. The flavohæmoprotein containing the DNA-component is now known as type I-cytochrome b_2, and that free of the DNA-component as type II-cytochrome b_2 (ref. 8). As observed by Appleby and Morton[6], the DNA-free enzyme does not crystallize when dialysed for short periods under similar conditions to those used for crystallization of the DNA-containing enzyme.

Unless otherwise specified, all procedures described in this article were carried out under essentially anaerobic conditions with oxygen-free solutions in a cold room at 0°–2° C. (see ref. 3).

To separate the DNA-component from type I-cytochrome b_2, a solution containing about 26 mgm./ml. of three-times crystallized protein[3] was dialysed successively (for 15–24 hr. in each case) against 50 per cent and 70 per cent saturated solutions of ammonium sulphate at *p*H 8·0 (refs. 6 and 7). The precipitated protein was collected by centrifuging and washed with a 70 per cent saturated solution of ammonium sulphate at *p*H 8·0. The combined supernatants contained the protein-free deoxyribonucleic acid component. The DNA-free flavohæmoprotein (type II-cytochrome b_2) was dissolved in a minimum amount of 0·5 *M* sodium lactate / 0·1 m*M* ethylenediamine tetraacetate / 0·04 *M* tetrasodium pyrophosphate, *p*H 6·8, centrifuged to remove a small amount of precipitate, and then dialysed against 0·05 *M* sodium lactate / 0·1 m*M* ethylenediamine tetraacetate / 1 m*M* magnesium sulphate at *p*H 6·8. There was no change in the dialysing solution for about 30 hr., at about which time turbidity appeared and increased gradually as dialysis was continued. After several days, large pink crystals were observed adhering to the sides and bottom of the 'Visking' cellulose sac. After 24 days, the crystals were collected and washed by allowing them to settle under gravity after suspending in 0·05 *M* sodium lactate / 0·1 m*M* ethylenediamine tetraacetate / 1 m*M* magnesium sulphate at *p*H 6·8. The size and density of the crystals created difficulties in collection and transfer; centrifuging was unnecessary. The shape of the larger crystals was recognizable without aid of a hand lens.

Whereas the crystals of type I (DNA-containing)-cytochrome b_2 obtained under similar conditions belong to the tetragonal system, and are probably shallow bi-pyramids which appear as square plates when lying flat (see refs. 1 and 3), the crystals of type II (DNA-free)-cytochrome b_2 belong to the hexagonal system and consist of hexagonal prisms with pyramids at both ends (Fig. 1). Fig. 2 shows measurements on average crystals of type I- and type II-cytochrome b_2. The largest crystals of type II-cytochrome b_2 were about three times the size of that illustrated in Fig. 2, but they were somewhat fragile and difficulty was experienced in handling them without chipping the faces or the tips of the pyramids. Crystals of type I-cytochrome b_2 are weakly and those of type II-cytochrome b_2 strongly anisotropic, especially when oriented in certain directions.

A pellet of crystals of type II-cytochrome b_2 appeared darker in colour than a similar pellet of type I crystals, probably due to the difference in size and structure of the crystals. Type II crystals

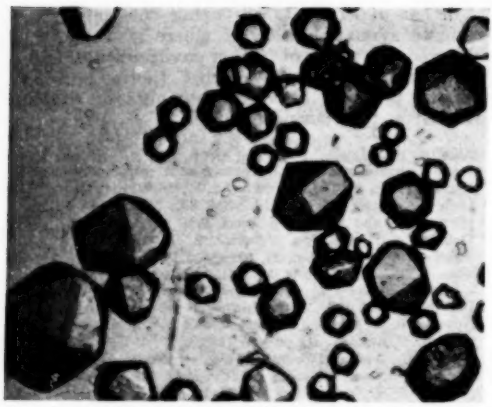
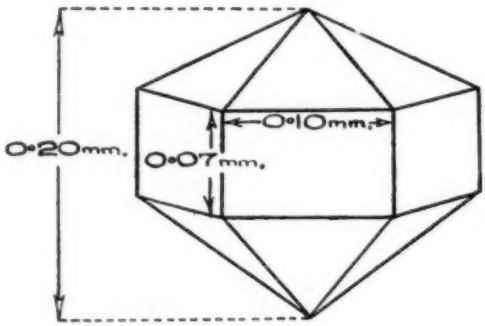


Fig. 1. Crystals of type II (DNA-free)-cytochrome b_2 (× 125)

Type I



Type II

Fig. 2. Dimensions of representative crystals of type I (DNA-containing)- and of type II (DNA-free)-cytochrome b_2 obtained under similar conditions

dissolved slowly in 0·5 *M* sodium lactate / 0·04 *M* tetrasodium pyrophosphate / 0·1 m*M* ethylenediamine tetraacetate, *p*H 6·8. On dialysis of this solution (containing about 35 mgm. protein/ml.), under the conditions used for first crystallization, crystallization was complete in a few hours; the crystals had the same form as in Fig. 1, but they were very much smaller. All crystals, both in the initial and recrystallized material, appeared to be perfectly formed. A number of preparations of crystalline type II-cytochrome b_2 have been produced as described.

Table 1 shows comparative properties of solutions of crystalline type I- and type II-cytochrome b_2. The enzymic and physico-chemical properties are very similar, but the absence of the DNA-component from type II material causes a change in the position of the maximum absorption and in the corresponding extinction value in the ultra-violet region of the spectrum. The absorption maximum at 269·5 mμ in type II-cytochrome b_2 appears to be due to the contribution of the riboflavin phosphate prosthetic group (which has an absorption maximum at 266 mμ) to the absorption due to the aromatic amino-acids; the flavin-free hæmoprotein derivative has a maximum at 278 mμ (refs. 8 and 9).

The two different crystalline forms of type I- and of type II-cytochrome b_2 are distinctive features of the DNA-containing and of the DNA-free enzyme. When a solution containing both types of cytochrome b_2 was dialysed as described for crystallization, both tetragonal plates (type I) and hexagonal prisms (type II) appeared in the precipitate. The larger and denser type II crystals were separated from type I crystals by forming a layer of a suspension of mixed crystals (in 0·05 *M* sodium lactate/0·1 m*M* ethylenediamine tetraacetate, *p*H 6·8) over 0·5 *M* sucrose / 0·05 *M* sodium lactate / 0·1 m*M* ethylenediamine tetraacetate at *p*H 6·8 and centrifuging at about 100*g* for 5 min. The type I crystals were almost completely retained in the uppermost layer, and type II crystals appeared in the precipitate. Whereas the solution of mixed type I and type II material had absorption maxima at 265 and 269 mμ, the solutions of the separated crystals showed the distinctive maxima of type I (265 mμ) and of type II (269·5 mμ) material.

The two crystal types may also be differentiated by fluorescence microscopy. When stained with an appropriate concentration of aqueous acridine orange, type I crystals show a strong green fluorescence due to the presence of the DNA component. The fluorescence is distributed uniformly throughout the crystal. With an excess of dye, an orange-red fluorescence is obtained, as with deoxyribonucleoproteins of the cell nucleus. However, type II crystals show no distinctive fluorescence on staining with acridine orange under the same conditions.

Type I (DNA-containing)-cytochrome b_2 is regarded as the native, naturally occurring form of the

Table 1. Comparative Properties of Crystalline Type I- and Type II-cytochrome b_2

Property	Type I	Type II
Specific enzymic activities*		
with heart-muscle cytochrome *c*	5,000	5,500
2 : 6-dichlorophenol indophenol ferricyanide	1,600 6,200	1,700 6,100
2 : 6-dichlorophenol indophenol plus phenazine methosulphate	3,800	4,100
Spectral properties of lactate-reduced compound†		
γ-band (423·5 mμ) / α-band (557·0 mμ)	6·3	6·3
β-band (528 mμ) / α-band (557·0 mμ)	0·5	0·5
γ-band (423·5 mμ) / δ-band (328 mμ)	4·6	4·0
ultra-violet band (265 or 269·5 mμ)‡ / γ-band (423·5 mμ)	0·86	0·55
Chemical properties		
DNA	5–6%	absent
molecules of riboflavin phosphate / molecules of protohæm	1	1
$S°_{20,w}$ (Svedbergs)§	8·45	8·17

* Enzymic activities were estimated with DL-sodium lactate at 25° C., as described by Appleby and Morton[3], from changes in extinctions measured for the first minute of the reaction. The concentration of 2 : 6-dichlorophenol indophenol was 0·07 m*M*, and of phenazine methosulphate was 0·07 per cent; the reactions with these dyes were carried out at *p*H 8·0. Values are for two preparations prepared and tested under identical conditions, but they are not necessarily the maximum values for either type of preparation.

† The ratios are based on extinctions measured at the appropriate absorption maxima with an Optica Model *CF*4 grating spectrophotometer; see Morton[10].

‡ The maximum absorption is at 265 mμ for type I and at 269·5 mμ for type II material.

§ $S_{20,w}$ was 7·74 for type I-cytochrome b_2 (55 μ*M* hæm) and 7·40 for type II-cytochrome b_2 (174 μ*M* hæm); the concentration dependence of the sedimentation coefficients are given in ref. 11.

enzyme. A number of derivative proteins have now been prepared[5,9]. Type II (DNA-free)-cytochrome b_2 is such a derivative, but the name 'cytochrome b_2' is retained for this material as well as for type I material since both have similar dehydrogenase activity. The nomenclature of other non-enzymically active derivatives is discussed elsewhere[8].

We thank the Effront Yeast Co. Pty., Ltd., Melbourne, for the gifts of baker's yeast for this work, B. Palk for microphotography and R. Connolly for assistance in the preparation of type I-cytochrome b_2 and in the determination of the sedimentation coefficients.

[1] Appleby, C. A., and Morton, R. K., *Nature*, **173**, 749 (1954).
[2] Appleby, C. A., and Morton, R. K., *Biochem. J.*, **71**, 492 (1959).
[3] Appleby, C. A., and Morton, R. K., *Biochem. J.*, **73**, 539 (1959).
[4] Boeri, E., Cutolo, E., Luzzati, M., and Tosi, L., *Arch. Biochem. Biophys.*, **56**, 487 (1955).
[5] Morton, R. K., *Soc. Biol. Chem., Ind. Silver Jubilee Souvenir*, 177 (1955).
[6] Appleby, C. A., and Morton, R. K., *Biochem. J.*, **75**, 258 (1960).
[7] Montague, M. D., and Morton, R. K., *Nature*, **187**, 916 (1960).
[8] Morton, R. K., *Nature* (in the press).
[9] Morton, R. K., Armstrong, J. McD., and Appleby, C. A., in *Hæmatin Enzymes, Canberra*, 1959, edit. Falk, J. E., Lemberg, R., and Morton, R. K., 501 (Pergamon Press, London, 1961).
[10] Morton, R. K., *Rev. Pure and App. Chem.*, **8**, 161 (1958).
[11] Armstrong, J. McD., Coates, J. H., and Morton, R. K., in *Hæmatin Enzymes, Canberra*, 1959, edit. Falk, J. E., Lemberg, R., and Morton, R. K., 569 (Pergamon Press, London, 1961).

POSSIBLE CAUSES FOR PRODUCTION OF ISOLEUCINE BY *STREPTOMYCES RIMOSUS*

By Dr. I. HORVÁTH, I. GADÓ and A. SZENTIRMAI
Research Institute for Pharmaceutical Industry, Budapest 7

IT was observed that *Streptomyces rimosus* (strain *BS*21) when cultivated on media containing threonine[1] or α-keto-butyric acid[2] produces both isoleucine and acetylethylcarbinol. According to refs. 3–5, the biosynthesis of isoleucine and valine appears to proceed along the same pathway. In spite of this fact no production of valine could be demonstrated in media containing serine or pyruvic acid. Work described here was carried out in order to elucidate the possible causes of this phenomenon.

Details concerning the methods and inocula used were published earlier[6,7]. Also the media used in the present work were prepared similarly to those used earlier: 1·5 per cent glucose, 0·013 per cent KH_2PO_4, 0·3 per cent sodium chloride, 0·002 per cent 'Tween 80', and 0·5 per cent glycine or any other appropriate amino-acid in an amount corresponding to that of glycine by amino nitrogen content. The *p*H before sterilization was 7·0. The media were prepared with tap-water. In experiments with washed mycelia two-day-old cultures were used. Washing was performed with distilled water. The final dilution of mycelia in the incubation mixture was 3–5 mgm./ml. dry mycelia.

The demonstration of the enzyme synthesizing α-acetolactic acid was as follows: 2-ml. samples contained 5–10 mgm. washed mycelia (dry weight), 0·1 m.mole pyruvic acid, 0·02 m.mole Na_2AsO_4, 0·3 m.mole magnesium sulphate. The 2-ml. samples were incubated at *p*H 7·0 for 2 hr. at 28° C. and the reaction was stopped by the addition of 0·2 ml. 50 per cent (v/v) sulphuric acid. After standing for 2 hr. acetoin was steam-distilled from the mixture and determined as described in ref. 8. When washed mycelium walls were used, arsenate was omitted and replaced by 0·02 m.mole *p*H 7·0 phosphate buffer.

In media where conditions favourable for production of isoleucine were present 0·7 mgm./ml. of valine was found to have an inhibitory action on the production of isoleucine. This inhibition appeared to be specific for valine as no other amino-acid (in similar concentrations of amino nitrogen) was found to have such an effect. Isoleucine itself in concentrations of 0·8 mgm./ml. also failed to inhibit production, while the usual amount of the same substance was produced in addition to the level added.

When examining the production of isoleucine by washed mycelia, it was found that the rate and extent of production were very much influenced by the medium used for growing mycelia (Fig. 1). Mycelia grown on a threonine-containing medium immediately started isoleucine production in the presence of threonine and glucose. The production was not influenced by 100 μgm./ml. final concentration of chloramphenicol. When mycelia grown on a glycine-containing medium were used, the production of isoleucine started only during the fourth hour of incubation and could be completely inhibited by the addition of chloramphenicol (100 μgm./ml.).

In incubation mixtures containing serine and glucose the mycelia grown on a threonine-containing medium were found to produce valine. The production was remarkably enhanced by the addition of chloramphenicol (100 μgm./ml.) (Fig. 2). A similar production of valine could never be observed when mycelia grown on media containing any other amino-acid tested were used.

Strain *BS*21 of *Streptomyces rimosus* contains a high level of constitutive serine- and threonine-deaminase (dehydrase). The next step of isoleucine synthesis from threonine is accomplished by the enzyme synthesizing α-acetolactic acid[9–16]. We succeeded in

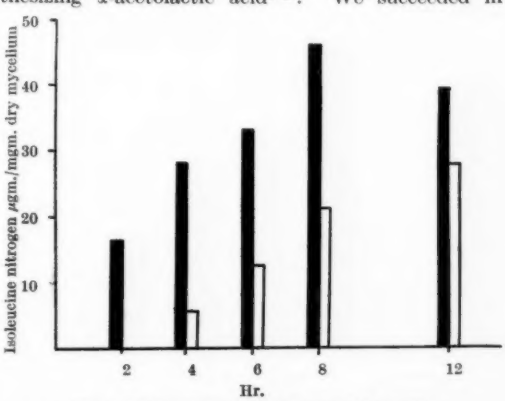


Fig. 1. Isoleucine production by washed mycelia. The incubation mixture contained 1 per cent glucose, 0·5 per cent DL-threonine, 0·3 per cent sodium chloride, 0·6 per cent KH_2PO_4 (*p*H adjusted to 7·0 by the addition of sodium hydroxide). Mycelium (dry weight) 4 mgm./ml.

Blacks columns, mycelia grown on threonine-containing medium; white columns, mycelia grown on glycine-containing medium

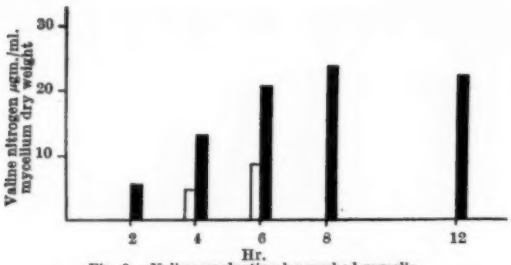


Fig. 2. Valine production by washed mycelia. The incubation mixture contained 1 per cent glucose, 0·5 per cent DL-serine, 0·3 per cent sodium chloride, 0·6 per cent KH_2PO_4 (*p*H adjusted to 7·0 by the addition of sodium hydroxide). Mycelium (dry weight) 5 mgm./ml.

White columns, no chloramphenicol; black columns, 100 μgm./ml. chloramphenicol added

demonstrating this enzyme in the presence of arsenite or arsenate and we found that the activity of the enzyme exhibited a five-fold increase when the micro-organism was grown on media containing threonine or α-keto-butyric acid (Table 1). Depression of this enzyme by valine in *E. coli* and *A. aerogenes* has been observed[12,14]. In our experiments such an effect of valine could be demonstrated similarly to the observations obtained with *Neurospora crassa*[16]. The enzyme was found to be bound to the surface of mycelia and the activity of mycelium walls (washed with 0·3 m.mole of magnesium sulphate, *p*H 7·0) obtained by ultrasonic disintegration was the same as that of intact mycelia. The former, however, was also active in the absence of arsenite or arsenate.

Table 1. Effect of the Medium on the Acetolactic Acid Synthesizing Enzyme Content of Mycelia

Medium* (per cent)	μgm. acetoin/mgm. mycelium/hr.
Glycine 0·5	3·6 (2·4– 4·8)
Glycine 0·5 + pyruvic acid 0·1	2·4 (1·8– 3·6)
DL-serine 0·7	3·0 (2·4– 4·2)
DL-threonine 0·79	13·2 (11·4–19·2)
DL-threonine 0·71 + DL-alanine 0·06	13·8 (10·2–20·4)
DL-threonine 0·71 + DL-valine 0·08	15·6 (12·0–22·2)
Glycine 0·5 + α-keto-butyric acid 0·1	10·8 (9·6–12·0)

* Basal medium: 1·5 per cent glucose, 0·013 per cent KH_2PO_4, 0·3 per cent sodium chloride, 0·002 per cent 'Tween 80'.

The results obtained can be explained as follows: The α-keto-butyric acid produced from threonine or added to the medium enhances inductively the activity of acetolactic acid and aceto-oxybutyric acid synthesizing enzyme and probably also that of other enzymes involved in valine and isoleucine synthesis, thus resulting in an increased production of these two amino-acids. Pyruvic acid did not seem to have a similar effect. The cleavage of these two amino-acids with a branching carbon chain is inductively enhanced by valine, resulting in the inhibition of isoleucine production, while chloramphenicol enhances for the same reason the production of valine by mycelia grown on a threonine-containing medium. The phenomenon observed, namely, the fact that an intermediary metabolite produced by one of the two amino-acids with similar pathways of biosynthesis or cleavage is able to enhance the biosynthesis or cleavage of both amino-acids is probably one of the general mechanisms controlling amino-acid metabolism.

Further details of the present work will be published in *Acta Microbiologica Hungarica*.

[1] Horváth, I., Gadó, I., and Szentirmai, A., *J. Bact.*, **78**, 293 (1959).
[2] Horváth, I., Gadó, I., and Szentirmai, A., *Acta Microbiol. Hung.* (in the press).
[3] Adalberg, E. A. *Amino Acid Metabolism*, 419, edit. Mc Elroy and Glass (Johns Hopkins Press, Baltimore, Maryland, 1955).
[4] Strassman, M., and Weinhouse, S., *ibid.*, 452.
[5] Umbarger, H. E., *ibid.*, 442.
[6] Horváth, I., Gadó, I., and Szentirmai, A., *Acta Microbiol. Hung.*, **5**, 317 (1958).
[7] Gadó, I., Szentirmai, A., Steczek, K., and Horváth, I., *Acta Microbiol. Hung.* (in the press).
[8] Westerfeld, W. W., *J. Biol. Chem.*, **161**, 485 (1945).
[9] Strassman, M., Thomas, A. J., and Weinhouse, S., *J. Amer. Chem. Soc.*, **77**, 1261 (1955).
[10] Strassman, M., Thomas, A. J., Locke, L. A., and Weinhouse, S., *ibid.*, **78**, 228 (1956).
[11] Umbarger, H. E., Brown, B., and Eyring, E. J., *ibid.*, **79**, 2980 (1957).
[12] Umbarger, H. E., and Brown, B., *J. Biol. Chem.*, **233**, 1156 (1958).
[13] Wagner, R. P., Berquist, A., and Forrest, H. S., *J. Biol. Chem.*, **234**, 99 (1959).
[14] Halpern, Y. S., and Umbarger, H. E., *J. Biol. Chem.*, **234**, 3067 (1959).
[15] Lewis, K. F., and Weinhouse, S., *J. Amer. Chem. Soc.*, **80**, 4913 (1958).
[16] Radhakrishnan, E. N., and Snell, E. E., *J. Biol. Chem.*, **235**, 2316 (1960).

A SUBCELLULAR COMPONENT OF *CORYNEBACTERIUM DIPHTHERIAE* HAVING THE CAPACITY TO BIND ANTI-TOXIN

By Prof. M. YONEDA and M. MATSUDA

Research Institute for Microbial Diseases, Osaka University

ATTEMPTS to elucidate the intracellular events occurring during biosynthesis of toxin revealed that a subcellular component having the capacity to bind anti-toxin was formed in toxin-producing diphtheria cells, while no such binding was detectable with a similar component derived from the cells of non-toxinogenic, non-lysogenic strains *C7* and *C4* of *C. diphtheriae*. Examinations of the anti-toxin-binding capacity of various subcellular fractions by using a quantitative absorption test showed that the highest activity was always found in the sediment obtained by centrifugation at 14,500–100,000*g*, but there was no activity in the cell-wall preparation.

In this work we used a substrain of toxinogenic Park Williams No. 8, the toxinogenic strains *C7*(β) and *C4*(β) of *C. diphtheriae* and the *C7* and *C4* indicator strains from which they had been derived by infection with β-phage[1,2].

These organisms were grown at 35° C. with shaking in Pope's medium[3] under an optimal iron condition (0·08 μgm. iron/ml.) for production of toxin by the strains of Park Williams No. 8, *C7*(β) and *C4*(β). After shaking for 20 hr., toxin content of the culture filtrates of these organisms was found to be 180, 18 and 12 Lf./ml., respectively, while no toxin was detectable in those of *C7* and *C4*. When shaking was complete, the organisms were centrifuged out of the growth medium, washed in ribonucleoprotein buffer[4] three times, made up into thick suspension in the buffer and exposed to sonic oscillation at 10 kc./s.

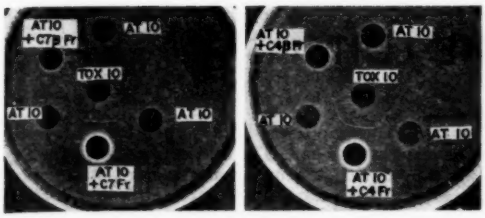


Fig. 1. Agar-gel precipitation pattern demonstrating anti-toxin binding by fraction/14,500–100,000 of *C7*(β) and of *C4*(β) of *C. diphtheriae*
TOX 10, 0·3 ml. of 10 Lf./ml. of crystalline diphtheria toxin; AT 10, 0·3 ml. of 10 units/ml. of a horse standard anti-toxin; AT 10 + *C7βFr*, *C4βFr*, *C7Fr* or *C4Fr*, 0·3 ml. of the supernatants of the mixtures of 0·6 ml. of 10 units/ml. anti-toxin and of 100 mgm. (dry weight) of fraction/14,500–100,000 of each organism

for 30 min. The whole disintegrates were then subjected to differential centrifugation as follows: the supernatants obtained from the disintegrates by centrifugation at low speed (5,000*g* for 30 min.) were centrifuged at 14,500*g* for 30 min. and the supernatants thus obtained, which were still slightly turbid, were finally centrifuged at 100,000*g* for 60 min. in a Spinco Model L. The sediments precipitated at 14,500*g* and at 100,000*g* and washed three times in ribonucleoprotein buffer by centrifugation at the same gravity were referred to as fraction/5,000–14,500 and fraction/14,500–100,000, respectively. Purified cell walls were provided by Prof. S. Kotani, of the Department of Bacteriology, Nara Medical College; these were prepared from toxin-producing cultures of Park Williams No. 8 strain by the method described by Kitaura *et al.*[5] in his laboratory.

Anti-toxin-binding capacity of these fractions and cell walls of Park Williams No. 8 cells were determined, using a quantitative absorption test. Thus, appropriate amounts of the materials corresponding to 70–400 mgm. of the dry weight were suspended in 15 ml. saline containing 15 units/ml. of a standard horse diphtheria anti-toxin, incubated at 37° C. for 15 min. and the supernatants obtained by centrifugation were measured for the residual content of

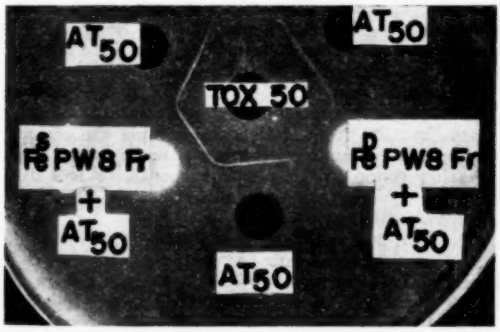


Fig. 2. Agar-precipitation pattern demonstrating the absence of anti-toxin-binding capacity in the fraction/14,500–100,000 derived from Park Williams No. 8 cells grown in the medium containing sufficient iron to suppress the toxin production
TOX 50, 0·3 ml. of 50 Lf./ml. of crystalline diphtheria toxin; AT_{50}, 0·3 ml. of 50 units/ml. of a horse standard anti-toxin; $Fe^{D}PW8Fr + AT_{50}$, 0·3 ml. of the mixture of 0·6 ml. of 50 units/ml. anti-toxin and of 125 mgm. (dry weight) of fraction/14,500–100,000 of cells of the cultures grown in an iron-deficiency for optimal toxin production
$Fe^{S}PW8Fr + AT_{50}$, 0·3 ml. of the mixture of 0·6 ml. of 50 units/ml. anti-toxin and of 125 mgm. (dry weight) of fraction/14,500–100,000 of cells of the cultures grown in the medium containing sufficient iron to suppress the toxin production

anti-toxin by flocculation with 15 Lf. diphtheria toxin. Anti-toxin-binding was also observed qualitatively with fraction/14,500–100,000 of each organism, using Ouchterlony's agar double-diffusion technique[6]. In this experiment, the supernatants obtained by centrifugation from the mixtures of the fraction and anti-toxin which had been incubated at 37° C. for 15 min., or the mixtures themselves were set against crystalline diphtheria toxin (3,300 Lf./mgm.) prepared by the method of Katsura *et al.*[7].

As can be seen from Fig. 1, while a slight difference was observed in the appearance of precipitation of toxin with anti-toxin regardless of the presence of *C7* or *C4* fractions, no precipitation lines were formed between the toxin well and those for anti-toxin plus the *C7*(β) or *C4*(β) fractions, indicating an absence or a great decrease of anti-toxin in the supernatants of the latter mixtures.

Table 1 shows the anti-toxin-binding capacity of various subcellular fractions obtained from Park Williams No. 8 cells. It may be seen that fraction/14,500–100,000 can bind as much as 0·68 unit of anti-toxin/mgm. of the dry weight. No activity was found in the purified cell wall preparation used. Of particular interest was the observation that similar fractions from the cells of Park Williams No. 8 strain which was grown in Pope's medium to which was added sufficient iron (8 μgm. iron/ml.) to suppress production of toxin showed no capacity to bind anti-toxin, as illustrated in Fig. 2.

Table 1. ANTI-TOXIN-BINDING CAPACITY OF VARIOUS CELLULAR FRACTIONS OF A PARK WILLIAMS NO. 8 STRAIN OF *C. diphtheriae*

Fractions	Anti-toxin absorbed (units/gm. dry weight)	
	Deficient iron*	Sufficient iron†
Fraction/5,000–14,500	256	0
Fraction/14,500–100,000	681	0
Purified cell wall	0	—

* Fractions of Park Williams No. 8 cells from the cultures grown in an iron-deficiency for optimal toxin production.
† Fractions of Park Williams No. 8 cells from the cultures grown in the medium containing sufficient iron to suppress toxin production.

Preliminary work showed that 1 mgm. (dry weight) of thoroughly washed fraction/14,500–100,000 of toxin-producing Park Williams No. 8 has no lethal action in guinea pigs, but gives the intradermal reaction to rabbits which is equivalent to 20 m.r.d. with a standard diphtheria toxin. The reaction can be neutralized by anti-toxin. Recent experiments have also shown that 0·8–5·0 units of anti-toxin can be formed in rabbits by immunizing with 5 mgm. (dry weight) of the washed fraction in incomplete Freund's adjuvants. Incidentally, fraction/14,500–100,000 can retain either the antigenicity or the original capacity to bind anti-toxin even after repeated washing (more than five times) in the ribonucleoprotein buffer or in other physiological buffers. This clearly indicates that the active principle must be in a bound form. Problems of relating subcellular structure to anti-toxin-binding capacity and the significance of the anti-toxin-binding principle in biosynthesis of diphtheria toxin still await further investigation.

[1] Barksdale, W. L., and Pappenheimer, A. M., *J. Bacteriol.*, **67**, 220 (1954).
[2] Groman, N. B., *J. Bacteriol.*, **69**, 9 (1955).
[3] Pope, C. G., and Linggood, F. V., *Brit. J. Exp. Path.*, **20**, 297 (1939).
[4] Gillchriest, W. C., *Microsomal Particles and Protein Synthesis*, 1 (Pergamon Press, London, 1958).
[5] Kitaura, T., Hirano, T., Inui, S., Kotani, S., Higashigawa, M., and Tsuchitani, T., *J. Nara Med. Assoc.*, **10**, 45 (1959).
[6] Ouchterlony, Ö., *Ark. Kemi. Mineral, Geol.*, **26**, 1 (1948).
[7] Katsura, T., Kato, I., Nakamura, H., and Koyama, J., *Japan. J. Microbiol.*, **1**, 213 (1957).

LETTERS TO THE EDITORS

GEOCHEMISTRY

Uranium in the Sikhote-Alin Meteorite and its Relation to the Lead Method of Age Determination

THE ages of lead of Earth and of meteorites are commonly based on the assumption that when these bodies were formed they contained lead of the same isotopic composition[1,2]. The present differences in the isotopic compositions of the lead in meteorites and also between common terrestrial lead and meteorite lead are due to radiogenic contributions from uranium and thorium since that time.

Recently, Starik *et al.*[3] measured the ratios of lead isotopes and the absolute amount of lead in a number of iron meteorites and found that the iron meteorites can be divided into two groups. One group has lead of 'primeval' isotopic composition, that is, the same as that measured by Patterson *et al.*[4] in the Canon Diablo meteorite. The other group has lead that is rich with isotopes lead-206, -207 and -208, relative to the isotope lead-204; this lead has an isotopic composition quite similar to that of common terrestrial lead (see Table 1). Reed *et al.*[5] have measured the uranium content of several iron meteorites and have found values in the range 10^{-10}–10^{-12} gm./gm. None of these meteorites, with the possible exception of Henbury, has lead enriched in the radiogenic isotopes. There is a dispute over the lead in Henbury[1,3].

Table 1. ISOTOPIC COMPOSITION OF LEAD IN THE SIKHOTE-ALIN AND CANON DIABLO METEORITES

Iron meteorite	Lead concentration	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
Sikhote-Alin	3×10^{-5}	17·89	15·84	38·19
	3×10^{-4}	17·55	15·60	37·97
Canon Diablo	—	9·46	10·34	29·44

In order to test the assumption that the excess of lead-206 is due to the radioactive decay of the isotope uranium-238, of lead-207 to that of uranium-235, and of lead-208 to that of thorium-232, we searched for uranium-235 in the Sikhote-Alin meteorite, which is a meteorite that clearly[3] has a lead-207 excess. We found less than $1{\cdot}4 \times 10^{-13}$ gm./gm. of uranium-235, corresponding to less than 2×10^{-11} gm./gm. of normal uranium. This amount is more than a factor of one hundred too small to account for the excess lead-207 in the Sikhote-Alin meteorite in 5×10^9 years.

We irradiated two 7-gm. samples of Sikhote-Alin for 9 hr., and one uranium standard for 2 hr., in a pneumatic tube of the Massachusetts Institute of Technology reactor. The flux was 7×10^{12} neutrons/cm.^{2} sec. We measured the fission product, xenon-135, which has a 9·2 hr. half-life. Since the xenon-135 follows iodine-135, which has a 6·7 hr. half-life in the fission chain, we waited about 12 hr. after the irradiation before processing the samples for xenon. The fission product xenon-133 has been used to measure uranium in stone meteorites[6]. In order to remove possible uranium contamination, we dissolved away 10 per cent of the irradiated samples in nitric acid. We placed the remainder in an alumina crucible in our gas extraction system and melted it by induction heating. The sample was kept molten for 1 hr. in the presence of carrier gas. The carrier used with the first sample and with the uranium standard was xenon; the carrier used with the second sample was a 50–50 mixture of xenon and argon. At the end of the heating, only several small droplets remained in the crucible; the rest of the material had evaporated to the walls of the crucible and the furnace. The hydrogen was pumped away from the gas by its diffusion through a hot palladium thimble. The remaining gas was kept over hot calcium (550° C.). The xenon yield from the first sample and from the uranium standard was 100 per cent. This xenon was taken from the calcium and put into a Geiger counter. For the second sample the gas was removed from the calcium by adsorption on charcoal at the temperature of liquid nitrogen. Argon was removed from the charcoal at 0° C. and xenon removed at 160° C. The xenon yield was 65 per cent; this xenon was added to a Geiger counter. The Geiger counter was removed from the extraction line and placed in an iron shield; the background rate was 15–20 counts/min. The uranium standard consisted of $3{\cdot}0 \times 10^{-8}$ gm. of normal uranium ($2{\cdot}1 \times 10^{-10}$ gm. of uranium-235) in the form of uranyl nitrate evaporated from solution on an aluminium foil. This sample was placed with 7 gm. of iron in an alumina crucible and subjected to the identical experimental procedure that was used with the first Sikhote-Alin sample. The xenon from the standard when first counted 6 hr. after the melting gave 315 ± 5 counts/min.; this activity, which we followed for several days, clearly decayed with a 9·2 half-life to 35 ± 5 counts/min. The amount of long-lived activity agrees with the amount of xenon-133 expected. These results correspond to 420 ± 20 counts/min. of xenon-135 at the time of melting. The gas from the first Sikhote-Alin sample gave 70 counts/min. of a long-lived activity; there was less than 15 counts/min. of 9-hr. activity at the time of melting. This negative result corresponds to less than $2{\cdot}8 \times 10^{-13}$ gm./gm. of uranium-235 or 4×10^{-11} gm./gm. of normal uranium. The long-lived activity was probably argon-37, since this amount of argon-37 could result from 3×10^{-8} c.c./gm. of argon-36 or 5×10^{-7} gm./gm. of calcium in the sample. In the xenon fraction from the second sample the activity was within 5 counts per minute of background. This negative xenon-135 result corresponds to less than $1{\cdot}4 \times 10^{-13}$ gm./gm. of uranium-235 or 2×10^{-11} gm./gm. of normal uranium.

The lead-207 excess in the Sikhote-Alin meteorite is $2{\cdot}2 \times 10^{-9}$ gm./gm. A period of 10^{10} years is required for this lead-207 excess to result from $1{\cdot}5 \times 10^{-13}$ gm./gm. of uranium-235. There is less than $1{\cdot}4 \times 10^{-13}$ gm./gm. of uranium-235 according to our measurements. Such a large age seems unreasonable since it would require that the uranium produced in nucleosynthesis were practically pure uranium-235. The uranium and lead contents of the Sikhote-Alin meteorite are inconsistent with the common assumption made in determining lead ages for Earth and for meteorites.

Starik *et al.* have postulated separate origins, each with a different 'primeval' isotopic lead composition, for the two groups of iron meteorites. This postulate

is consistent with the uranium results but makes ambiguous the interpretation of lead ages of meteorites and of Earth.

One of us (D. E. F.) is grateful to the U.S. Atomic Energy Commission for financial support, and to the Smithsonian Astrophysical Observatory for hospitality during the period in which this work was undertaken.

EDWARD L. FIREMAN

Smithsonian Astrophysical Observatory,
Cambridge, Massachusetts.

DAVID E. FISHER

Department of Engineering Physics,
Cornell University,
Ithaca, N.Y.

[1] Patterson, C., *Geochim. et Cosmochim. Acta*, **10**, 230 (1956).
[2] Masuda, A., *Geochim. et Cosmochim. Acta*, **13**, 143 (1958).
[3] Starik, I. E., Sobotovich, E. V., Lovtsyus, G. P., Shats, M. M., and Lovtsyus, A. V., *Sov. Phys. (Doklady)*, **134**, 555 (1960).
[4] Patterson, C., Brown, H., Tilton, G., and Inghram, M., *Phys. Rev.*, **92**, 1234 (1953).
[5] Reed, G. W., Hamaguchi, H., and Turkevich, A., *Geochim. et Cosmochim. Acta*, **13**, 248 (1958).
[6] König, H., and Wänke, H., *Z. Naturforsch.*, **14**, a, 866 (1959).

GEOPHYSICS

Palæomagnetism of Hawaiian Lava Flows

PALÆOMAGNETIC investigations of volcanic rocks extruded in various parts of the world during the past several million years have generally revealed a younger sequence of lava flows magnetized nearly parallel to the field of a theoretical geocentric axial dipole, underlain by a sequence of older flows with exactly the opposite direction of remanent magnetization. A 180-degree reversal of the geomagnetic field, occurring near the middle of the Pleistocene epoch, has been inferred by many workers from such results[1-3]. This is a preliminary report of an investigation of 755 oriented samples collected from 152 lava flows on the island of Hawaii, selected to represent as many stratigraphic horizons as possible. (Sampling details are indicated in Table 1.) This work was undertaken because Hawaii's numerous thick sequences of lava flows, previously mapped as Pliocene to Historic by Stearns and Macdonald[4], and afterwards assigned ages ranging from later Tertiary to Recent, by Macdonald and Davis[5], appeared to offer an ideal opportunity to examine the most recent reversal of Earth's field.

Remanent magnetization measurements using a spinner type magnetometer have now been completed on at least one sample from each of the 152 lava flows sampled; flow averages of all measurements completed to date before demagnetization are shown in Figs. 1, 2 and 3. Demagnetization experiments of representative samples in alternating fields up to 800 œrsteds (peak) typically reduce the intensity of magnetization by 90 per cent but do not significantly change directions of magnetization. Statistical analyses of the direction of magnetization in the samples, before and after demagnetization, and mineralogical studies are in progress.

The preliminary measurements may be summarized as follows: (1) The historic flows have directions of magnetization closely grouped about the present geomagnetic field direction on Hawaii, which has changed less than 2 degrees since about 1900. (2) None of the 152 flows sampled has reversed magnetic polarity. (3) The scatter in directions of magnetization from flow to flow in a given sequence is much smaller than generally reported in comparable studies elsewhere. (4) The average magnetization directions of most of the thick sequences sampled appear to differ significantly both from the present geomagnetic field and from the theoretical field of an axial dipole; moreover, the average directions in different sequences are different from each other.

If the geomagnetic field reversal theory is true, then all the flows sampled on Hawaii are post-reversal and hence younger than early Pleistocene. Alternative explanations (*a*) that lavas were not extruded when the field was reversed or were afterwards eroded or buried, or (*b*) that self-reversal occurred only in lava flows extruded while Earth's field was reversed, are much less plausible. Moreover, the stratigraphic distribution of the 152 lava flows sampled renders unlikely the possibility that a reversely magnetized sequence is present but was inadvertently missed.

The closely parallel directions of magnetization found throughout these rather thick sequences of flows may be interpreted in two ways: (1) An entire sequence may have been extruded in a time-interval too short for large amounts of secular variation to have occurred. However, rates of secular variation as determined from direct measurements at observatories commonly cause more angular variation in several hundred years than is typically observed in the Hawaiian sequences. Accumulation within several hundred years of sequences of more than 50 clearly distinguishable lava flows with total thicknesses up to 450 ft. appears rather unlikely. (2) That low rates of secular variation prevailed in Hawaii during the time these lava flows were extruded is a more plausible explanation. The results from the Historic flows support this interpretation, and the direct measurements back to about 1900 also show less secular variation than is typical elsewhere in the world.

Table 1. SAMPLING DETAILS OF VOLCANIC SERIES EXAMINED

Geological Unit	Age	Flows sampled	Stratigraphic thickness (ft.)	Description
Historic flows	Historic	9	—	Extruded at known historical dates between 1750 and 1955; members of Kau, Hualalai, and Puna Volcanic Series are included (Fig. 1*a*)
Puna Volcanic Series	Late Pleistocene (?) and Recent	18	373 (I) 64 (II)	From Uwekahuna Bluff on west wall of Kilauea caldera. Group II constitutes the lowest 64 ft. of the section, and is separated from Group I by an erosional unconformity (Fig. 1*b*).
Kahuku Volcanic Series	Pleistocene	29	99 (I) 188 (II) 54 (III)	Stratigraphically, flow Groups I, II and III are progressively lower (Fig. 2*a*)
Hamakua Volcanic Series	Late Tertiary and Quaternary	27	135	From south-east side of Laupahoehoe Canyon. These flows comprise the lower members of the Hamakua Volcanic Series, as described by Stearns and Macdonald (ref. 4) (Fig. 2*b*)
Pololu Volcanic Series	Pliocene (?)	50	453	From Waipio Valley. These flows are from the lower member of the series; the highly weathered upper member was not sampled (Fig. 3*a*)
Ninole Volcanic Series	Pliocene (?)	25	116+	From Kaiholena Ridge. The upper 76 ft. were sampled on the east side of the ridge, the lower 40 ft. on the west; an unknown thickness between these is not exposed (Fig. 3*b*)

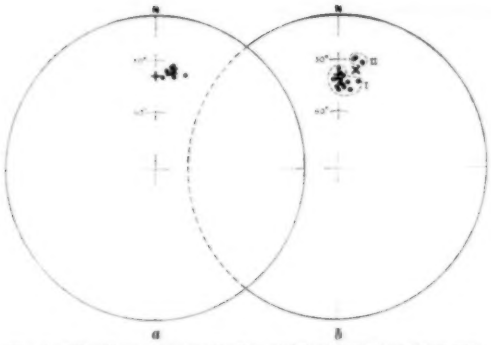


Fig. 1. Directions of magnetization of (*a*) Historic lava flows and (*b*) Puna Volcanic Series. ●, North-seeking polarity downwards; ○, north-seeking polarity upwards; +, direction of axially symmetric dipole field in Hawaii; ×, present geomagnetic field direction in Hawaii. (Lambert equal-area projection with prime circle horizontal)

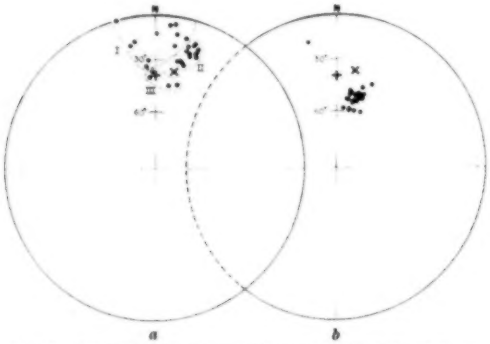


Fig. 2. Directions of magnetization of (*a*) Kahuku Volcanic Series and (*b*) Hamakua Volcanic Series. Symbols as for Fig. 1

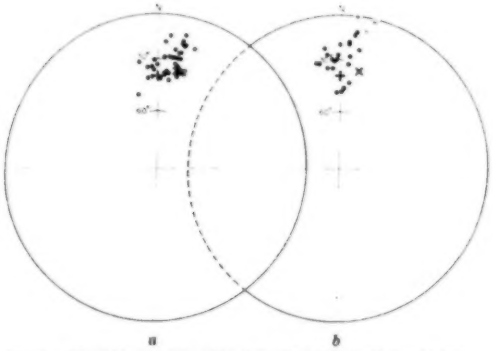


Fig. 3. Directions of magnetization of (*a*) Pololu Volcanic Series and (*b*) Ninole Volcanic Series. Symbols as for Fig. 1

The differences observed between average magnetization directions of the different sequences of flows and the axial dipole field direction do not, in our opinion, indicate that Hawaii has undergone displacements and rotations in the Pleistocene. Such an interpretation would require: (*a*) lower latitudes than at present for Hawaii during the extrusion of the Pololu and Ninole Volcanic Series; (*b*) markedly higher latitudes during the extrusion of the Hamakua Series; (*c*) lower latitudes during Kahuku Series volcanism; (*d*) the present latitude during extrusion of the Puna Series; substantial rotations of the island of Hawaii since the early Pleistocene would also be required. Much more plausible is the explanation that each of these sequences spans a time-interval too short to give an average direction of magnetization parallel to an axial dipole field. Although absolute lengths of time cannot be given, it appears from this investigation that stratigraphic sections 450 ft. thick and composed of more than 50 flows may accumulate on shield volcanoes in periods of time so short that the non-axial dipole field components do not vectorially add to zero. Conversely, the geomagnetic field may have non-axial dipole components for times long enough for the accumulation of these relatively thick sections.

Publication of this communication is authorized by the Director of the U.S. Geological Survey.

Richard R. Doell
Allan Cox

U.S. Geological Survey,
Menlo Park,
California.

[1] Matuyama, M., *Proc. Imp. Acad. Japan*, **5**, 203 (1929).
[2] Hospers, J., *Nature*, **168**, 1111 (1951).
[3] Roche, A., *C.R. Acad. Sci., Paris*, **243**, 812 (1956).
[4] Stearns, H. T., and Macdonald, G. A., Hawaii Div. Hydrography, *Bull.* **9** (1946).
[5] Macdonald, G. A., and Davis, D. A., "Zone des Hawaï", Chap. 2 in *Océanie proprement dite*, Fasc. 2, 6. *Océanie—Lexique stratigraphique internationale*, by Intern. Geol. Cong., Strat. Comm., 71 (Centre Nat. Recherche Sci., Paris, 1956).

PHYSICS

Emission of Ultra-Violet and Visible Radiation from Metals by the Impact of High-Energy Hydrogen Ions

In a recent communication[1] we reported that ultra-violet and visible radiation is emitted from nickel by the impact of high-energy protons and positive ions, and that the visible part of the spectrum is continuous. With a resolved proton beam we observed a flat peak between 4600 and 4900 Å. in the distribution curve of spectral intensity.

Apart from the radiation resulting from neutralization of positive ions on metal surfaces[2], the point of most significance is to investigate if the radiation observed by us contains spectral lines or the spectrum of the target element on which high-energy positive ions impinge. If these lines (or spectrum) are present, their origin remains to be traced.

Townes[3] has developed a theory of sputtering of metals under positive ion bombardment based on the assumption of local heating. Kapitza[4] has explained in a similar way the emission of electrons from metal surfaces by positive ions. This theory is supported by the experimental work of Oliphant and others[5], but some theories[6] of sputtering fail to account for recent experimental results. The mechanism of sputtering of metals by positive ions is indeed not yet well understood.

We wish to consider the probable mechanism of the emission of radiation from the target metal. There seem to be two possibilities for the emission of visible and ultra-violet spectra of the target element: (1) the radiation may be emitted from sputtered atoms excited in gaseous phase by positive ions, or by the secondary electrons knocked out of the target; (2) the excited atoms giving out this radiation may originate in the metal itself by the impact

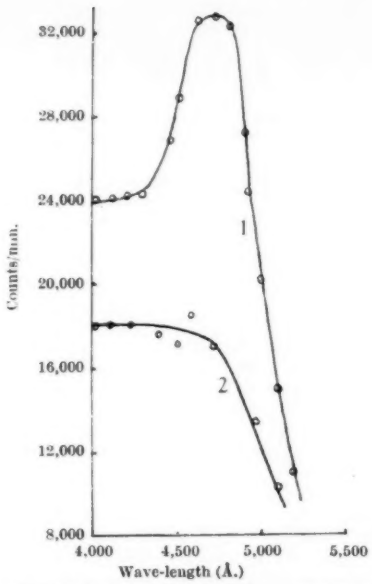


Fig. 1. Spectral intensity of distribution curve for radiation emitted from nickel by high-energy positive ions of hydrogen. Energy of positive ions = 200 keV.; positive ion current = 4 μamp. Curve 1, target cooled to low temperature; curve 2, target not cooled

of high-energy positive ions. It is difficult to distinguish which of these two processes predominates in the emission of this radiation.

If the metal target is cooled to a very low temperature where its thermal conductivity is comparatively high, the local heating effect will be reduced and consequently the sputtering will be less. If the radiation observed were due to sputtered atoms excited in the gaseous phase, its intensity would be smaller at lower temperatures of the target. Our experimental results, however, show that the reverse is the case.

The target is placed in a special chamber made throughout of glass. It houses, besides the target, a copper reservoir filled with liquid air, so that impurities and volatile vapours in the chamber may condense on it. The nickel target is degassed with electron bombardment to a bright red heat under a high vacuum for a sufficiently long time to make it clean and free from gas. It is then cooled and kept permanently in thermal contact with a metal reservoir maintained at liquid-air temperature during experiments, and radiation emitted from it under the impact of hydrogen ions of energy 200 keV. and current strength 4 μamp. is studied with Kodak gelatine filters. Filter 2*B* is used to cut out the ultra-violet region. The distribution curve of spectral intensity shows a pronounced peak between wave-lengths 4600 and 4800 Å., which is a region of strong lines in the arc spectrum of nickel. Curves 1 and 2 of Fig. 1 show the distribution of spectral intensity obtained with the target cooled to low temperature and without cooling it respectively. The visible spectrum is, so far as could be judged from analysis with gelatine filters, continuous within the range studied. The intensity of the ultra-violet radiation between 4000 and 3300 Å. is greater than that of the visible region as in previous experiments.

The presence of the peak in the region of prominent nickel lines in curve 1 leads us to think that this radiation is emitted from the excited atoms which originate in the metal itself under the impact of high-energy positive ions. The continuous nature of the spectrum, on the other hand, suggests that some sort of electron plasma oscillations may occur in the metal due to positive ion bombardment on it, and may give rise to this radiation.

We believe that, with our experimental arrangement, the chances of accumulation of impurities on the cooled target are small; and consequently the increase in the intensity of the radiation emitted from the cold metal surface cannot be attributed wholly to the excitation of atoms of any dirty film on it.

Details of the chamber and the target cooling arrangement are being described elsewhere.

We are grateful to Sir George Thomson for suggestions and advice, and to Sir Mark Oliphant for his interest throughout this work.

Rafi Mohammed Chaudhri
Mustafa Yar Khan

High Tension and
Nuclear Research Laboratory,
Church Road,
Government College,
Lahore, West Pakistan.

[1] Chaudhri, R. M., and Khan, M. Y., *Nature*, **189**, 996 (1961).
[2] Oliphant, M. L. E., *Proc. Roy. Soc.*, A, **124**, 228 (1929).
[3] Townes, C. H., *Phys. Rev.*, **65**, 319 (1944).
[4] Kapitza, P., *Phil. Mag.*, **45**, 981 (1923).
[5] Oliphant, M. L., and Moon, P. B., *Proc. Roy. Soc.*, A, **27**, 388 (1930). Chaudhri, R. M., and Khan, A. W., *Proc. Phys. Soc.*, **61**, 526 (1948).
[6] Harrison, D. E., *Phys. Rev.*, **102**, 1473 (1956).

High-Speed Direct-Recording System for Electron Diffraction

An electron diffraction system for the quantitative investigation of rapid phase changes in solids has recently been completed. The system will record an electron diffraction pattern of the Debye–Scherrer ring type in 50 msec., giving the electron intensity at the peaks of strong reflexions to an accuracy of 2 per cent. The system gives an improvement in speed of direct recording of about four orders of magnitude[1], and is thus attractive for investigating quantitatively rapid changes in solids such as polymorphic transformations, transitions from the amorphous to the crystalline state, diffusion in alloys. It is also suitable for investigating controversial questions in electron diffraction such as the transition from geometrical to dynamic diffraction conditions[2,3].

Fig. 1*a* shows a typical record from a polycrystalline gold film about 300 Å. thick. There are in fact two superimposed traces each of 100-msec. duration showing profiles of rings from 220 to beyond 531. The display also shows the profile of the primary beam after it has traversed the film; this gives a continuous measure of the angular resolution of the electron optical system. Once the information in the diffracted beams has been obtained at a sufficient resolution and level it is, of course, easy to magnify the diffracted profiles on the display. Thus Fig. 1*b* shows the inner rings of the gold together with the main beam in greater detail.

Figs. 2*a* and *b* show a high-speed amorphous-to-crystalline change in a film of silver in response to sudden heating. Fig. 2*a* is the envelope of fifty traces, the first of which shows almost no rings while

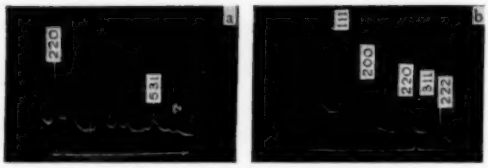


Fig. 1

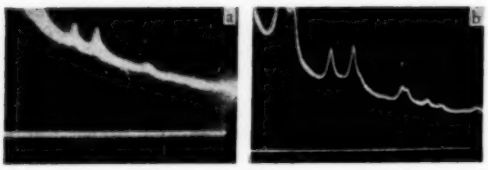


Fig. 2

the last trace, 5 sec. later, shows the rings considerably developed. After 200 sec. (Fig. 2*b*) there is little further change.

The ultimate speed of recording in such a system is set by shot noise in the diffracted beams. The limitations are the same as those of the scanning electron microscope[4]. Measurements show that the intensities at diffracted peaks are about 10^{-9} amp. Thus, for an accuracy of 1 per cent at such a peak, a picture point may be recorded in about 2 μsec. One may expect that recording times for complete diffraction patterns of 1 msec. are a question merely of improvements in technology.

C. W. B. Grigson

Department of Engineering,
University of Cambridge.

[1] Bagdyk'yants and Alekseev, *Bull. Acad. Sci. U.S.S.R.*, Phys. Ser., **23**, 766 (1958).
[2] Pinsker, *Adv. in Electronics and Electron Physics*, **11**, 355 (1959).
[3] Halliday, *Proc. Roy. Soc.*, A, **254**, 30 (1960).
[4] Smith and Oatley, *Brit. J. App. Phys.*, **6**, 391 (1955).

Isotopic Composition of Atmospheric Hydrogen and Methane

In a recent communication, Bishop and Taylor[1] express the opinion that the tritium concentration of free hydrogen in the atmosphere has been rising over the past ten years, with a doubling time of approximately 18 months. The authors suspect that artificial tritium was released into the atmosphere several years before the Castle test series in 1954, which is commonly assumed to have led to the first pronounced rise in the tritium concentration of terrestrial surface water. Bishop and Taylor's communication includes a diagram of the logarithms of all the experimentally determined tritium values in free atmospheric hydrogen plotted against time. The plot shows that the values follow a straight line that includes the first value obtained by Faltings and Harteck[2] on atmospheric hydrogen collected in 1948.

The question of an addition of artificial tritium into the atmosphere prior to 1954 is of considerable importance in connexion with estimating the natural rate of production by cosmic rays of tritium, which is in general assumed to be of the order of 1 tritium atom per sec. per cm.^{2} of Earth's surface.

We have been able to secure a sample of atmospheric hydrogen, dating from 1948, contained in 'raw neon' in a regular cylinder, through the courtesy of Mr. F. R. Balcar of the Air Reduction Co., New York. This gas gave the following values:

T/H = $3{\cdot}5 \pm 0{\cdot}1 \times 10^{-14}$ ≡ 35,000 tritium units
δD = $-5{\cdot}4 \pm 0{\cdot}2$ per cent relative to Lake Michigan water standard

This tritium concentration is very close to that found by Begemann in hydrogen collected shortly before the Castle tests in 1954, and to the value for 1954 represented by the straight line through the points measured by several investigators afterwards. It therefore appears unlikely that artificial tritium has influenced significantly the tritium content of atmospheric hydrogen prior to 1954. The deuterium value shows, in agreement with previous findings[3–4], that equilibrium between atmospheric water vapour and hydrogen is not established at ordinary temperatures.

A most remarkable observation was recently made by Bishop *et al.*[5]. The tritium content of atmospheric methane is also very high and only about a factor of 5 below that of present-day atmospheric hydrogen. The authors state that they are unable to suggest any satisfactory explanation for the source of this tritiated methane. They suspect an artificial origin.

We have independently investigated one sample of atmospheric methane, which was collected in January 1960, by the Linde Co. in Gary, Indiana. This sample was large enough to enable us to carry out very accurate, routine carbon-14 and tritium analyses in the La Jolla Radio-carbon and Tritium Laboratories. We are not able to propose any satisfactory explanation for the following experimental findings, but hope that our results, which include deuterium and carbon-13 values, may help in the future in interpreting the observed facts. Measurement of carbon-13 was carried out in the laboratory of H. Craig (University of California, San Diego), who wishes to discuss his result more extensively in a separate paper. A second tritium determination by F. Begemann at Mainz gave exactly the same result as ours.

T/H = $1{\cdot}94 \pm 0{\cdot}1 \times 10^{-14}$ ≡ 19,400 tritium units
^{14}C = $75{\cdot}2 \pm 0{\cdot}5$ per cent of standard wood, or 60 per cent of atmospheric carbon dioxide in 1960
δD = $-4{\cdot}2 \pm 0{\cdot}2$ per cent relative to Lake Michigan standard water
$\delta^{13}C$ = $-39 \pm 0{\cdot}1$ per mille relative to Chicago Pee Dee Belemnite standard (ref. 6).

The tritium value of our 1960 sample is exactly the same as the average of the samples from the northern hemisphere collected by Bishop *et al.* during 1958 and 1959, although tritium in terrestrial surface water showed wide variations during this period. A comparison of our carbon-14 value with results obtained by W. F. Libby on atmospheric methane from 1952 shows no change greater than experimental errors. Libby's samples contained 75 per cent and 60 per cent of modern carbon. They were collected in Britain by the British Oxygen Co., at Wembley, and submitted in the form of carbonate by H. E. Gluckauf. We are grateful to Dr. Libby for his unpublished results.

Agreement of the results is better than might be expected in view of the possibility that artificial inactive methane and hydrogen from city gas, automobile exhaust, and other industrial sources might be present. Limits on the amounts of such artificial gases present can be deduced from the measured value of the stable isotopes. We are planning to make at least one measurement a year in the future.

Support for this work was received from the U.S. Atomic Energy Commission Division of Biology and Medicine, from the National Science Foundation and

from the Geophysics Directorate of the Air Force Cambridge Research Center.

This communication is published with the permission of the Director, U.S. Geological Survey.

A. E. Bainbridge
H. E. Suess
Scripps Institution of Oceanography,
University of California,
San Diego, La Jolla, California.
Irving Friedman
U.S. Geological Survey,
Washington, D.C.

[1] Bishop, K. F., and Taylor, B. T., *Nature*, **185**, 26 (1960).
[2] Faltings, V., and Harteck, P., *Z. Naturforsch.*, **5**, a, 438 (1950).
[3] Harteck, P., and Suess, H. E., *Naturwiss.*, **36**, 218 (1949).
[4] Begemann, F., and Friedman, I., *Z. Naturforsch.*, **14**, a, 1024 (1959).
[5] Bishop, K. F., Delafield, H. J., Eggleton, A. E. G., Peabody C. O., and Taylor, B. T., Paper No. *TTS*/79, *I.A.E.A. Symp. on Detection and Use of Tritium in the Physical and Biological Sciences*, Vienna (1961).
[6] Craig, H., *Geochim. et Cosmochim. Acta*, **12**, 133 (1957).

Our communication on the growth of the tritium content of free hydrogen in the atmosphere was written before the publication by Begemann and Friedman[1] of detailed measurements for the period 1954–56. These showed considerable seasonal variation, but no pronounced increase during the two years following the Castle test series. Their earliest determination in January 1954 before the Castle test series was $9{\cdot}18 \times 10^4$ tritium units compared with $1{\cdot}66 \pm 0{\cdot}097 \times 10^4$ tritium units found by Grosse *et al.*[2] in the spring of 1952. This obvious increase was concluded by Begemann and Friedman to be due to the Ivy test series of November 1952. Evidence for an earlier increase based on the value $0{\cdot}38 \pm 0{\cdot}125 \times 10^4$ tritium units obtained by Faltings and Harteck[3] in 1948–49 now appears to be refuted by the value of $3{\cdot}5 \times 10^4$ tritium units reported by Bainbridge, Suess and Friedman. It may be noted, however, that this value is more than twice that observed by Grosse in 1952. With three sets of observations indicating a substantial increase prior to the Castle tests, it is difficult to support the conclusion of Bainbridge *et al.* that such an increase is "unlikely".

Our measurements[4] of the tritium content of atmospheric methane collected at Wembley by the British Oxygen Company gave values ranging from $1{\cdot}59 \times 10^4$ to $3{\cdot}36 \times 10^4$ tritium units during the period October 1958 to February 1959, the average value being $2{\cdot}19 \times 10^4$ tritium units. One sample collected on December 7, 1959, had a value of $2{\cdot}85 \times 10^4$ tritium units and another collected on February 8, 1960, a value of $1{\cdot}59 \times 10^4$ tritium units. It is true that the present reported value ($1{\cdot}94 \times 10^4$ tritium units) falls within the range of values obtained by us, but to state that it "is exactly the same" as the average of our samples collected in the northern hemisphere during 1958 and 1959 may give the incorrect impression that short-term fluctuations are very small.

Furthermore, in addition to the considerable short-term fluctuations observed at Wembley, we found that the levels in the northern hemisphere stratosphere and in the southern hemisphere generally were all about $0{\cdot}8 \times 10^4$ tritium units, considerably lower than those at Wembley. The level in the southern hemisphere troposphere has also shown a continuous rise during 1953–58 when our measurements ceased.

It is clear, therefore, that though the future measurements proposed by Bainbridge *et al.* will give some useful information, a more comprehensive sampling programme would be necessary to unravel the origin and distribution of tritiated methane in the atmosphere.

K. F. Bishop
B. T. Taylor
A. E. J. Eggleton
Health Physics Division,
Atomic Energy Research Establishment,
Harwell, Didcot.

[1] Begemann, F., and Friedman, I., *Z. Naturforsch.*, **14**, a, 1024 (1959).
[2] Grosse, A. V., Kirshenbaum, A. D., Kulp, J. L., and Broecker, W. S., *Phys. Rev.*, **93**, 250 (1954).
[3] Harteck, P., *J. Chem. Phys.*, **22**, 1746 (1954).
[4] Bishop, K. F., Delafield, H. J., Eggleton, A. E. G., Peabody, C. O., and Taylor, B. T., Paper No. *TTS*/79, *I.A.E.A. Symp. on Detection and Use of Tritium in the Physical and Biological Sciences*, Vienna (1961).

ENGINEERING

A Novel Electrostatic Machine: the Corona Motor

We would like to direct the attention of readers to a new type of electrostatic motor which may find use in some specialized application. The operation of the motor demonstrates one of the fundamental properties of electrostatic fields, namely, motion of charge under the influence of a voltage gradient.

The motor is shown in Fig. 1 and consists of a disk, made from an insulating material, which is mounted on a spindle, and three pairs of electrodes mounted circumferentially around the disk. The electrodes are alternately connected to the positive and negative terminals of a source of high-voltage direct current. The diameter of the disk is 1·5 in., and a supply of 8–13 kV. is sufficient to operate the motor. The disk rotates either clockwise or anti-clockwise, depending on the direction in which it was started. When running freely in air with the axis turning in jewel bearings, speeds of up to 12,000 r.p.m. are attained.

During running of the motor a corona discharge takes place along the circumference of the disk and extends from each positive electrode towards the next negative electrode against the direction of rotation. The glow discharge increases in intensity and extent with increase in voltage until at a critical voltage flashover occurs between the electrodes.

We surmise that the operation of the motor can be explained as follows (Fig. 2): Consider that the voltage

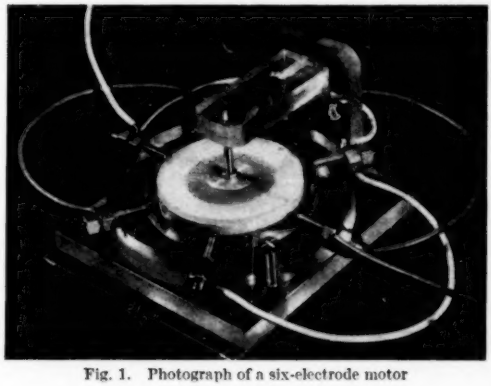


Fig. 1. Photograph of a six-electrode motor

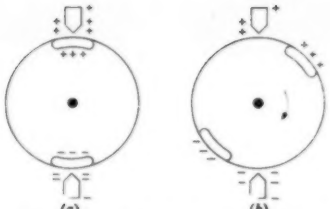


Fig. 2. *a*, Charge is deposited on the rim of the disk by ionization of the air gap. *b*, Disk rotates under action of torque developed by charge dipole

on the electrodes is increased until ionization of the air between the electrodes and the rim of the disk occurs, then a quantity of charge will be deposited on the rim (Fig. 2(*a*)). The charge, being of the same polarity as the electrode from which it emanated, is mechanically in unstable equilibrium with respect to the electric field, and if the disk is slightly disturbed, a couple will act on the disk, which will cause it to rotate, carrying the charge with it (Fig. 2 (*b*)). On reaching the opposite electrode, the charge is neutralized by a further ionized path in the air gap. If the process of charging and discharging carries on continuously, the disk will continue to rotate and will be able to deliver torque.

A possible explanation of why a glow discharge is noticed between alternate pairs of electrodes only is that a negative electrode deposits electrons on the disk, which creates an electric field between the charged area and the positive electrode. When the charged area is carried into the region where the resultant field intensity is large enough, ionization takes place. The positive electrodes, on the other hand, act as collectors of the negative charge. This results in a glow discharge between pairs of negative and positive electrodes only. At low voltages the ionization takes place close to the positive electrode and the region is extended for increased applied voltage until it covers the entire area between positive and negative electrodes. This is in conformity with the mechanism suggested, since the ionization will take place at a given field strength and thus the distance that separates the charged surface from the positive electrode, when ionization starts, will be determined by the magnitude of the charge.

In order to study the wave-forms of current when the motor is running, a small resistance was inserted in the earth lead, and the probe of an oscilloscope was connected across this resistance. It was found that charge transport takes place in short discrete bursts, of approximately 1 μsec. duration. The number of discharges a second depends on the speed of rotation, but a typical value is 2,000 discharges a second. Fig. 3 shows the wave-form occurring in a two-electrode motor.

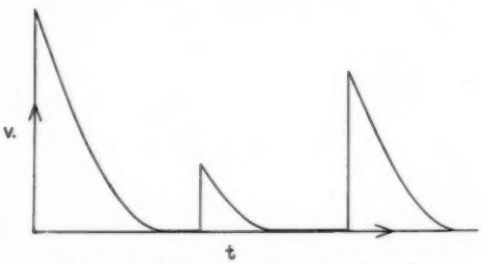


Fig. 3. Current wave-forms of a two-electrode motor

The corona motor is the converse of the Van de Graaf generator. In both cases charge is the active medium, but while in the Van de Graaf generator it is transported against an electric field to supply voltage, in the motor it is allowed to move with the field in order to supply torque.

The simplicity of construction and the absence of magnetic and metallic material might well justify further research to develop the motor into an efficient unit.

We wish to thank Mr. E. Fiedeldey for the mechanical construction of the device.

J. D. N. Van Wyk
G. J. Kühn

Electrical Engineering Division,
National Physical Research Laboratory,
South African Council for Scientific
and Industrial Research,
Pretoria.

Rectifying Action of Non-uniform Fields in Dielectrics

Preliminary work has been done on the breakdown of dielectric specimens under direct and 50 c./s. alternating voltages. With the latter a polarity detector was connected in series with the test specimen and in all cases point–plane electrode systems were used. For air and transformer oil a nickel-plated steel

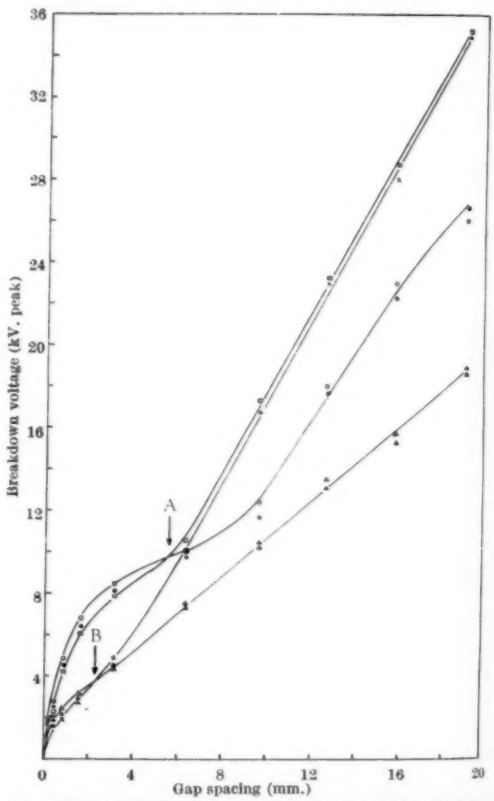


Fig. 1. Direct-current and 50 c./s. alternating-current breakdown voltage characteristics of air. Brass rod: □, negative point (d.c.); ○, positive point (d.c.); ●, 50 c./s. (a.c.). Nickel-plated point: ×, negative point (d.c.); △, positive point (d.c.); ▲, 50 c./s. (a.c.)

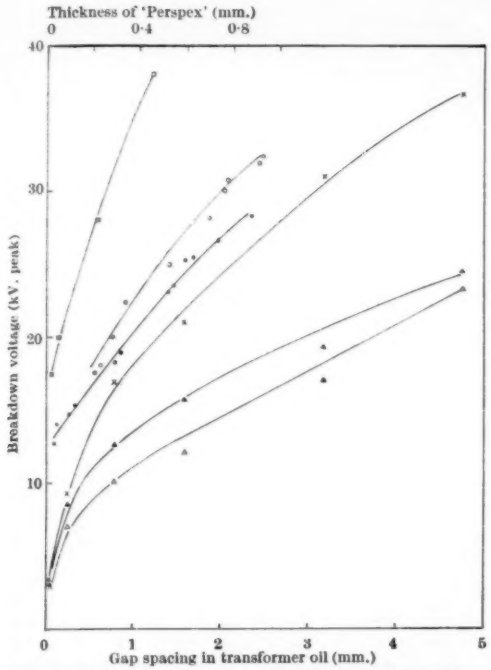


Fig. 2. Direct-current and 50 c./s. alternating-current breakdown voltage characteristics of 'Perspex' and transformer oil. Electrodes: nickel-plated steel point–plane. 'Perspex': □, negative point (d.c.); ○, positive point (d.c.); ●, 50 c./s. (a.c.). Transformer oil: ×, negative point (d.c.); △, positive point (d.c.); ▲, 50 c./s. (a.c.)

sewing needle having a radius of curvature of about 2·5μ and a $\frac{1}{2}$-in. diameter brass rod having a radius of curvature of 1/16 in. were used in conjunction with a brass plate as the plane electrode. For 'Perspex' sheets the point electrode, a sewing needle similar to that described above, was pressed into the specimen at a temperature of 155° C. and an aluminium foil was bonded to the other side of the specimen as the plane electrode. This assembly was then immersed in transformer oil. All measurements were made under normal temperature and pressure conditions.

It was observed that in 50 c./s. alternating fields breakdown of air always occurs when the point is at the positive half-cycle provided that the gap spacing is greater than the critical spacing shown at *A* and *B* in Fig. 1 for the two different point electrodes. The peak value of the alternating-current breakdown voltage is practically equal to the direct-current breakdown voltage with the point positive. Breakdown under alternating voltages of either positive or negative polarity only occurs when the gap spacings are close to the critical value. This polarity effect or rectifying phenomenon with large gap spacings is independent of the rate of rise of the applied voltage.

This phenomenon also occurs in transformer oil and 'Perspex' as shown in Fig. 2. Within the range of gap spacings under investigation the breakdown always occurs when the point is at the positive half-cycle. In these two dielectrics other effects apart from the space charges may play an important part in the breakdown mechanisms. The fact that the peak value of the alternating-current breakdown voltage in transformer oil is higher than the direct-current breakdown voltage with the point positive may be attributed to the effect of impurities. The reversed tendency noted in 'Perspex' may be due to the thermal effect.

The consistency of this phenomenon depends on the non-uniformity of the field, and the rectifying action, especially when air is used as the dielectric medium, can possibly be used to rectify high-voltage alternating currents.

I wish to thank the Brush Electrical Engineering Co., Ltd., for permission to publish this communication.

K. C. Kao

Research Division,
Brush Electrical Engineering Co., Ltd.,
Loughborough, Leics.

CHEMISTRY

Experimental Detection of Auto-Ionizing Transitions in Krypton by Electron Impact

A NUMBER of investigators[1] have discussed the contribution of auto-ionization to the shape of the ionization efficiency curves obtained by electron impact; but, at least in the case of the rare gases, no direct correlation has been found between the structure observed in these curves and the energy-levels known to be strongly auto-ionizing from ultra-violet absorption spectroscopy[2]. I wish to present ionization efficiency curves for the Kr^+ ion which exhibit discontinuities in slope at electron energies that lie quite close to the levels found in absorption. Both the intensity and separation of these levels fall off rather rapidly at higher energies, so that only those that lie directly above the normal $^2P^0_{3/2}$ ionization-level can be expected to be resolved. Using the Paschen notation[3] for clearness in the figures; these levels are:

5 S'_1, 14·07 eV. 6 S'_1, 14·25 eV. 7 S'_1, 14·37 eV.
4 S_2, 14·09 eV. 5 S_2, 14·27 eV. 6 S_2, 14·37 eV.

Three independent determinations of the ionization efficiency curve for the Kr^+ ion are shown in Fig. 1. This figure is a photograph of the original recorder traces with the co-ordinate lines filtered out. The curves were obtained by the retarding potential method[4], which had been modified by the addition of a.c. signal detection, and an x–y recorder to obtain a continuous plot of the 'difference' ion current as a function of the electron energy. The terms 'response 2', 'response 3', 'response 4' indicate that successively larger capacitance was introduced between the mechanically rectified output of the a.c. amplifier and the x–y recorder to improve the over-all signal to noise ratio. The scale for the 'difference' ion current (ordinate) is arbitrary and the divisions are unmarked since the zero of the y-axis amplifier was shifted between determinations to permit all three curves to be traced out on the same sheet of co-ordinate paper. The electron energy scale has been shifted approximately 0·3 eV. to place the onset of ionization to the $^2P^0_{3/2}$ level at its spectroscopic value of 14·0 (13·996) eV. The electron energy was varied by a motor-driven helipot, and a signal proportional to it was impressed on the x-axis amplifier of the recorder. A photographic enlargement of Fig. 1 showing the onset of ionization and the break in the vicinity of the unresolved 5 S'_1 and 4 S_2 levels is given in Fig. 2, and a similar enlargement showing the break near the 6 S'_1 and 5 S_2 levels in Fig. 3. No clear break is to be found in the neighbourhood

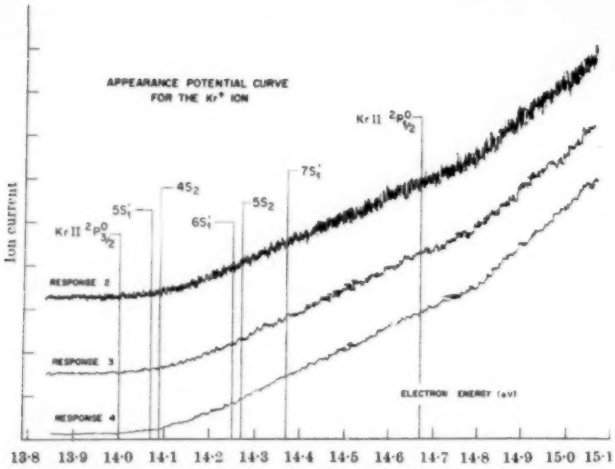


Fig. 1. Appearance of potential curves and auto-ionizing levels for the Kr^+ ion

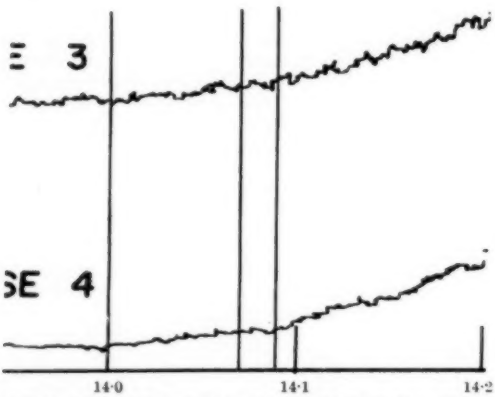


Fig. 2. Enlarged section of Fig. 1 showing the breaks at onset and near the location of the 5 S'_1 and 4 S_2-levels

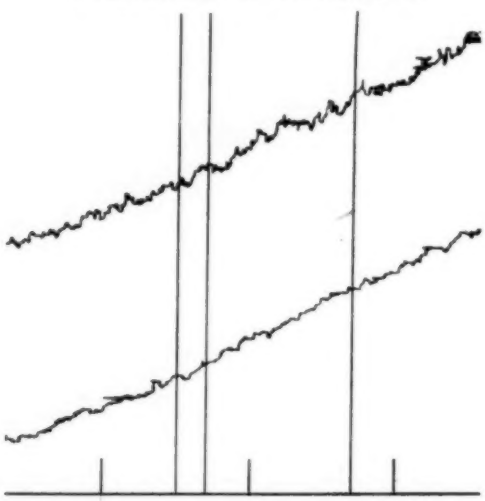


Fig. 3. Enlarged section of Fig. 1 showing the break near the 6 S'_1 and 5 S_2-levels

of the 7 S'_1 level on the curves presented in Fig. 1, and the curve labelled response 2 was not enlarged because of the high noise-level.

These three curves were obtained a little more than a year ago for use in a paper describing the modification of the retarding potential method, which permitted the ionization efficiency curve to be plotted directly on the recorder paper as a continuous function of the electron energy. The toe of the curve was not examined critically at that time and the departure of the curve from linearity at about 0·15 eV. above onset was a source of considerable disappointment. It was attributed to excessive curvature resulting from an abnormally large spread in the electron energy introduced by the square-wave modulation of the electron beam required for a.c. signal detection of the 'difference' ion current. Every effort was made to remove this 'curvature' by adjusting the position of the source magnet, the retarding potential, electrode voltages, etc., but it was not found possible to reduce it below that appearing in Fig. 1.

Following the publication of a paper by Foner and Nall[5] on the structure found in the ionization efficiency curves of the rare gases, the curves shown in the figure were re-examined and the structure discussed earlier was found. Only a few other curves had been preserved, but on nearly all of these a break of some sort could be found near the first two pairs of auto-ionizing levels. These breaks were rather easily identified on five other ionization efficiency curves in addition to those shown in the graphs. Three of these latter showed a fairly strong indication of a break near the 7 S'_1 level. Since the measurements of these curves extended over a period of months and were obtained under different adjustments of the focus of the source, it seems hard to dismiss the agreement between these electron impact results and the spectroscopic data as accidental or instrumental. A more detailed report will be prepared as soon as the mass spectrometer used in this work becomes available again.

I thank Prof. W. R. S. Garton and Lady Anne Pery-Thorne for a very helpful discussion of auto-ionizing processes in general during a visit to the Imperial College of Science and Technology last autumn, and to Prof. Garton for pointing out that the auto-ionizing levels listed above as well as many others have been tabulated.

John F. Burns

Analytical Chemistry Division,
Oak Ridge National Laboratory*,
Oak Ridge,
Tennessee.

* Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

[1] Fox, R. E., Hickam, W. M., Kjeldaas, jun., T., *Phys. Rev.*, **89**, 555 (1953). Dorman, F. H., Morrison, J. D., and Nicholson, A. J. C., *J. Chem. Phys.*, **32**, 378 (1960).

[2] Beutler, H., *Z. Phys.*, **93**, 177 (1935). Pery-Thorne, Anne, and Garton, W. R. S., *Proc. Phys. Soc.*, **76**, 833 (1960).

[3] Moore, C. E., *Atomic Energy Levels*, National Bureau of Standards Circular **467**, 2.

[4] Fox, R. E., Hickam, W. M., Kjeldaas, jun., T., and Grove, D. J., *Phys. Rev.*, **84**, 859 (1951); *Rev. Sci. Instr.*, **26**, 1101 (1955).

[5] Foner, S. N., and Nall, B. H., *Phys. Rev.*, **122**, 512 (1961).

Surface Properties of Silver Iodide

It is a rather curious fact that both low-energy and high-energy surfaces abound, but intermediate energy surfaces of high area are infrequently found and have been little studied. In a search of such intermediates, we recently examined the surface chemistry of silver iodide. One method is to compare the apparent water specific surface area to the nitrogen area. Another result which reflects the surface energy is the measurement of the heat of immersion in water and other liquids of particles of the solid sufficiently high in surface area to give meaningful results.

Interest in the surface chemistry of silver iodide increases when one comes to recognize that silver iodide is regarded as hydrophobic[1] in classical colloid chemistry but as hydrophilic in cloud physics[2,3]. When the double layer on silver iodide sols is repressed or destroyed, the colloid chemist finds that the particles flocculate quickly and settle out. Yet this result may be due only to gravity and might have nothing to do with any hydrophobic character. Previous results on the water vapour adsorption on silver iodide are quite contrary. Birstein[3] claims some 130 layers of water are adsorbed before P_0 is reached. It can be categorically stated that such a reported result is novel. Solids adsorb at most 5–10 nominal layers before condensation is reached, as Harkins[4] showed years ago. In a recent paper, Dubinin *et al.*[5] also concluded that the silver iodide surface is hydrophilic and that it accepts about 3 layers before the condensation pressure.

The cloud physicists regard silver iodide as the best 'cloud seeder' yet found presumably because its crystal parameters closely correspond to those of ice. Observations of the nucleation and growth of ice crystals on single silver iodide crystals (colour motion pictures taken by Prof. B. J. Mason, Imperial College, London) cast doubt on the concept of epitaxy. For one thing, the ice crystals preferentially form at steps in the silver iodide crystal face where epitaxy cannot exist in all directions. For another, the hexagonal pattern does not appear until the growing ice crystal has gained some size. Finally, many ice crystals are formed on a single silver iodide crystal so that the epitaxy does not extend over the entire face as in the usual case.

Some doubt was cast on the other direct measurements of the hydrophilic character of silver iodide by Halsey[7] in a brief note on the matter. He concluded that ice and silver iodide surfaces are energetically incompatible although he was not considering cloud seeding at the time. He was freezing xenon on to various crystals to attempt to produce energetically uniform adsorbent surfaces.

Recent work in this Laboratory supports the contention that the silver iodide surface is rather hydrophobic. For one preparation, the value of the ratio of the Brunauer–Emmett–Teller areas for water to nitrogen surface area measurements were: $\Sigma H_2O/\Sigma N_2 = 7/100$, for another, $\Sigma H_2O/\Sigma Ar = 37/100$. For still another, $\Sigma H_2O/\Sigma N_2 = 27/100$. These results compare with the $\Sigma H_2O/\Sigma N_2 = 1/1{,}500$ obtained[8] for the almost completely hydrophobic graphitized carbon black, 'Graphon'.

Further work is in progress to try to establish the surface characteristics of silver iodide. For this purpose, high surface area samples in sizeable quantity are desired. These are difficult to prepare, and then to outgas without severe sintering[7].

Our present hypothesis is that the steps in the silver iodide surface possess hydrophilic, possibly oxide, sites. On these the water molecules first adsorb in clusters much as on 'Graphon' or graphites. Eventually, when the nucleation process is carried out at low temperatures, the ice crystal structure develops.

It seems reasonable to suspect higher sticking coefficient for the water on to earlier adsorbed molecules if they are not highly organized and 'busy' interacting strongly with the substrate. This situation would probably prevail if water molecules could be adsorbed all over the surface as on an oxide. This possible explanation of the efficacy of silver iodide is strengthened by the recent work of Eyring[9] on the sticking coefficient of many atoms and molecules on a number of surfaces. He finds that the more ordered the molecules are in the surface, the lower is the sticking coefficient. The molecules do not want to pay the 'entropy price' and thus more of them rebound.

Possibly clays are good seeders because of the ion exchange sites which also build up water clusters; and proteins, reported by Vincent Schaefer to have excellent cloud seeding properties, are effective because of the separated polar sites at which water clusters can develop.

We wish to thank the National Science Foundation for a grant in support of this work.

A. C. Zettlemoyer
Noubar Tcheurekdjian
J. J. Chessick

Surface Chemistry Laboratory,
Lehigh University,
Bethlehem, Pennsylvania.

[1] Overbeek, J. Th. G., in *Colloid Science*, edit. by Kruyt, H. R., 1 (Elsevier Pub. Co., Amsterdam, 1952).
[2] Mason, B. J., *The Physics of Clouds* (Oxford Univ. Press, London, 1957).
[3] Birstein, S. J., *J. Meteorol.*, **12**, 324 (1955).
[4] Harkins, W. D., *The Physical Chemistry of Surface Films*, Chap. 3 (Rheinhold Pub. Corp., 1952).
[5] Moskvitin, N. N., Dubinin, M. M., and Sarakhov, A. I., *Izvest. Akad. Nauk. S.S.R., Otdel, Khin, Nauk*, **12**, 2080 (1959).
[6] Vonnegut, B., *J. App. Phys.*, **18**, 593 (1947).
[7] Karasz, F. H., Champion, W. M., and Halsey, jun., A. D., *J. Phys. Chem.*, **60**, 376 (1956).
[8] Young, G. J., Chessick, J. J., Healy, F. H., and Zettlemoyer, A. C. *J. Phys. Chem.*, **58**, 313 (1954).
[9] Eyring, H., and Wanlass, F. M., *Abstr. Papers, Div. Colloid and, Surface Chem. Amer. Chem. Soc.* (St. Louis, Missouri. March 1961).

Production of 'Active' Magnesium Oxide by heating in Vacuum

When samples of precipitated magnesium hydroxide were heated in air for a fixed period of 2 hr. at a succession of temperatures (T° C.) Gregg and Packer[1] found that the specific surface area S (determined by the Brunauer–Emmett–Teller method with nitrogen at − 183° C.) when plotted against T gave the curve I reproduced in Fig. 1.

Conducting a similar experiment with starting material similarly prepared and having a similar specific surface (60 $m.^2$ $gm.^{-1}$ as compared with 56 $m.^2$ $gm.^{-1}$), but heating *in vacuo*, we obtained curve II of Fig. 1: the temperature at which the maximal area was obtained has been lowered from *c.* 390° C. to *c.* 300° C. and the maximal area has been raised from 190 $m.^2$ $gm.^{-1}$ to 340 $m.^2$ $gm.^{-1}$, which is unusually high for 'active' magnesium oxide.

This effect of heating in air as compared with vacuum is qualitatively similar to that reported by Razouk and Mikhail[2] working with the mineral brucite, but their areas were considerably lower, and the

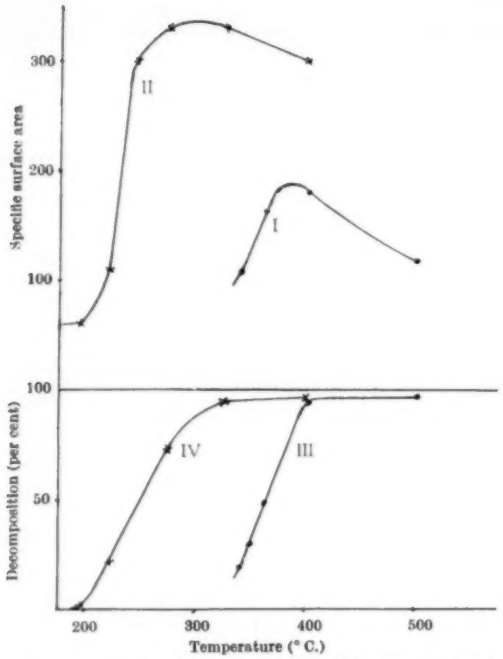


Fig. 1. Calcination of magnesium hydroxide in air (curves I and III, see ref. 1) and *in vacuo* (curves II and IV, present work)

maximum was situated at a higher temperature (air, maximal area, 37 m.^{2} gm.$^{-1}$, at 500° C.; vacuum, maximal area, 124 m.^{2} gm.$^{-1}$, at 350° C.). This is possibly due to the larger crystallite size of the mineral brucite as compared with precipitated magnesium hydroxide.

An explanation of the superior 'activating' effect of heating in vacuum must await the results of experiments now in progress in this laboratory, but it is probably connected with the higher rate of decomposition *in vacuo*, and the reduction in sintering because of the lower temperature required for nearly complete decomposition (see curves III and IV).

R. Valverde
J. P. Sáenz

Falcultad de Química,
General Flores 2124,
Montevideo, Uruguay.

[1] Gregg, S. J., and Packer, R. K., *J. Chem. Soc.*, 51 (1955).
[2] Razouk, R. I., and Mikhail, R. Sh., *J. Phys. Chem.*, **61**, 888 (1957).

Catalytic Decomposition of Ethylene on Nickel

The mechanism of the hydrogenation of ethylene on nickel has been studied intensively for at least thirty years; but there is still little agreement as to the exact nature of the intermediates present on the metal surface during the reaction. On the other hand, examination of the chemisorption and dissociation of ethylene on nickel in the absence of gaseous hydrogen has been slight and mostly confined to ambient temperatures[1,2]. This is surprising as the products and mechanism of ethylene dissociation can play an important part in hydrogenation, exchange and other reactions.

The decomposition of ethylene on nickel has been studied as a function of temperature in the range −30° C. to 200° C. 14·8 gm. of high-purity carbonyl nickel were loosely pressed into pellets ¼ in. in diameter, reduced in a stream of hydrogen at 400° C. for 6 hr. and then evacuated at 400° C. for 12 hr. to a residual pressure of 1×10^{-6} mm. Experiment showed that no hydrogen remained on the metal after this treatment. 6 mm. of purified ethylene were then allowed to stand over the catalyst for a period of 4 hr. at each of the temperatures studied, the volume of the system being about 130 c.c. At the end of this period, the contents of the gas phase were condensed in a trap cooled in liquid nitrogen. The non-condensable pressure was found to be due to methane. The contents of the trap were analysed with a gas-chromatograph, using helium as carrier gas and a silica gel column. The results are shown in Fig. 1, in which the composition of the gas phase is plotted against the temperature of the catalyst.

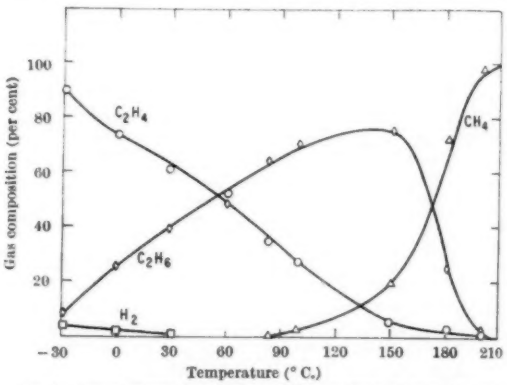


Fig. 1. Temperature-dependence of the products of reaction of ethylene (6 mm. pressure) with nickel after 4 hr. Gas concentrations: ○, C_2H_4; ◇, C_2H_6; △, CH_4; □, H_2

At temperatures below 60° C., the products are ethane and hydrogen, obtained by self-hydrogenation of the ethylene. This effect has been studied at 20° C. by Rideal and Jenkins[1], who suggest that ethylene in the gas phase reacts with chemisorbed hydrogen, obtained by dissociative adsorption of ethylene:

$$CH_2 = CH_2 \rightarrow \underset{*}{CH} = \underset{*}{CH} + \underset{* *}{HH}$$

The self-hydrogenation of ethylene was further studied by Beeck[2], who states that at −39° C. no ethane was produced. In the present work, however, appreciable quantities of ethane were found after reaction at −30° C. and the rate of self-hydrogenation was found to be measurable down to −78° C. The quantity of hydrogen evolved on self-hydrogenation was always small and less than 5 per cent of the ethane produced. No C_3, higher hydrocarbons or polymerization products were detected at low temperatures; this result is at variance with that of Morikawa *et al.*[4], who found 29 per cent butane among the products at 0° C. Above 60° C., methane began to appear in the gas phase due to the cracking of ethylene and ethane present; the concentration of the latter decreased rapidly above 150° C. and, at 200° C., the gas phase consisted entirely of methane.

The overall composition of the adsorbed residues on the surface can be calculated in the form $(CH_n)_x$, as shown in Fig. 2 At 0° C., these residues have an

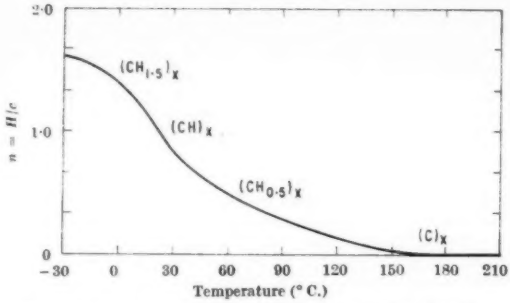


Fig. 2. Temperature-dependence of overall composition of surface residues $(CH_n)_x$

average composition corresponding to $(CH_{1\cdot5})_x$, but the value of n decreases rapidly with increasing temperature as hydrogen is progressively removed from the surface. These observations agree with those of Rideal and Jenkins[1], who found that the residues had an average composition at 20° C. approximating to that of acetylene, or $n = 1$. However, Pickering and Eckstrom[5] found no infra-red evidence for the presence of C—H bonds on the nickel surface under these conditions. Sabatier[6] found that complete carbonization of nickel occurred with ethylene at 300° C.; but it appears from the present work that the metal surface is essentially free of hydrogen at 200° C. All adsorbed residues could be completely removed and the metal restored in activity by the reduction and evacuation procedure described here.

DOUGLAS W. MCKEE

General Electric Research Laboratory,
Schenectady,
New York.

[1] Rideal, E. K., and Jenkins, G. I., *J. Chem. Soc.*, 2490 (1955).
[2] Eischens, R. P., and Pliskin, W. A., *Adv. in Catalysis*, **10**, 1 (1958).
[3] Beeck, O., *Rev. Mod. Phys.*, **17**, 61 (1945).
[4] Morikawa, K., Trenner, N. R., and Taylor, H. S., *J. Amer. Chem. Soc.*, **59**, 1103 (1937).
[5] Pickering, H. L., and Eckstrom, A. C., *J. Phys. Chem.*, **63**, 512 (1959).
[6] Sabatier, P., *La Catalyse en Chimie Organique* (Paris, 1920).

Role of Keto-Enol Tautomerism in the Polarographical Reduction of Some Carbonyl Compounds

DURING some polarographical investigations of certain α-aminoketones[1] and on the elimination of Mannich bases[2] experimental data were gathered, which can be explained by the occurrence of keto-enol transformation. Though considerable attention has been paid to the mechanism of polarographical reduction of carbonyl compounds[3], no experimental evidence has been adduced for the participation of the keto-enol tautomerism in the reduction of the carbon–oxygen bond. This follows from the fact that, in most cases examined, the keto form is the predominating form, and in buffered solutions used, establishment of keto-enol tautomeric equilibrium is fast. However, the decrease of the limiting current of some keto acids has recently been interpreted[4] as being influenced by the keto-enol equilibrium in addition to hydration. Experimental evidence for the role of enolization in the behaviour of substances like acetyl and acetonyl acetone[5] is unconvincing. On the other hand, it has been discovered that the enolate form participates

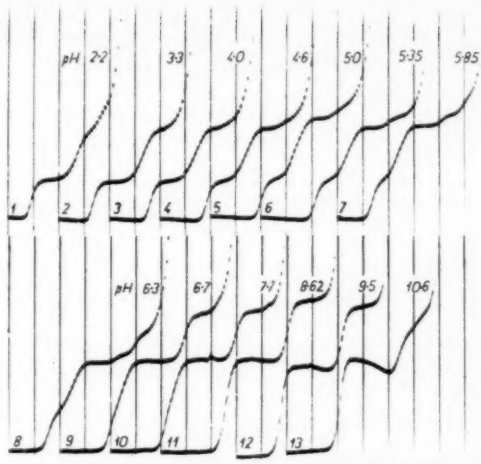


Fig. 1. Dependence on pH of waves fo rphenyl vinyl ketone. Britton–Robinson buffers, 1×10^{-4} M phenyl vinyl ketone. Curves starting at: 1–6, −0·4 V.; 7–11, −0·6 V.; 12 and 13, −0·8 V., 200 mV. (absc.), saturated calomel electrode, $h = 56$ cm., $t_1 = 3{\cdot}0$ sec., $m = 3{\cdot}3$ mgm./sec., full-scale sensitivity, 2·0 μamp.

when anodic waves occur, corresponding to the formation of mercury salts, in alkaline solutions of enolizable ketones (Heyrovský, M., private communication). Keto-enol equilibria were involved also in the complicated and, in some ways, questionable scheme derived[6] for the reduction of thenoyl trifluoroacetone.

Since the conditions for detecting keto-enol tautomerism in solutions are unfavourable, we have focused attention on cases where the carbonyl compound is formed electrolytically at the surface of the electrode.

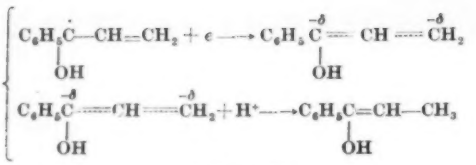
As an example of such an electrode process the reduction of phenyl vinyl ketone is first mentioned. In this case (Fig. 1) a two-electron reduction occurs first in two one-electron steps in acid solution, followed by waves at more negative potentials. The half-wave potentials of the latter are practically identical with those for propiophenone. The participation of keto-enol tautomerism is deduced from the dependence of the height of this more negative wave on pH and buffer composition. It is assumed that irreducible enol formed as a reduction product in the first two-electron step is transformed by an acid-base catalysed reaction into the reducible keto form. The rate of formation of the keto form from the enol form governs the limiting current of the more negative wave, which acquires a kinetic character. The assumed irreducibility of the enol form is in agreement with the behaviour of stilbenes. Stilbene itself is reduced at −2·3 V. and the introduction of a hydroxyl group could result ($\rho^* \doteq +0{\cdot}30$ V., $\sigma^I_{OH} = +0{\cdot}25$) in a shift of maximum by +0·1 V.

Hence the mechanism of reduction can be depicted as follows:

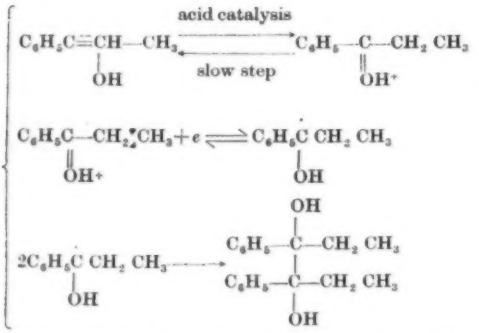
$p\mathrm{H} < 2$

$$\left.\begin{array}{l} \mathrm{C_6H_5C(=OH^+)-CH=CH_2} + e \rightleftharpoons \mathrm{C_6H_5\dot{C}(OH)-CH=CH_2} \\ \\ 2\,\mathrm{C_6H_5\dot{C}(OH)-CH=CH_2} \longrightarrow \text{Dimer} \end{array}\right\} \; 1{\cdot}1$$

1·2

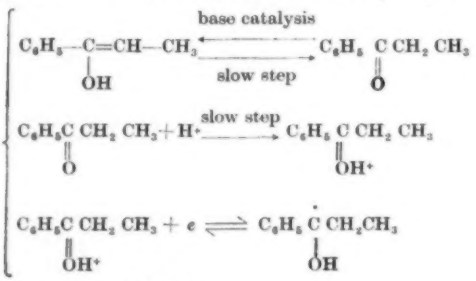


2·1



*p*H 4–6 Waves 1·1 and 1·2 identical as for *p*H < 2.

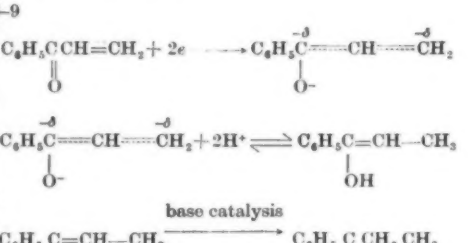
2·1′



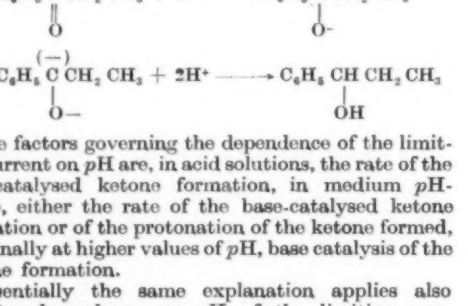
and dimerization as in 2·1

*p*H 6–9

1·3



2·3



The factors governing the dependence of the limiting current on *p*H are, in acid solutions, the rate of the acid-catalysed ketone formation, in medium *p*H-range, either the rate of the base-catalysed ketone formation or of the protonation of the ketone formed, and finally at higher values of *p*H, base catalysis of the ketone formation.

Essentially the same explanation applies also for the dependence on *p*H of the limiting currents of acetophenone, when it is formed from $C_6H_5CO\,CH_2—X$ (*X* = halogen, NR_3^+, O*R*, S*R*, etc.) observed by Zuman and Horák[1] and Lund[2]. The enolate ion $C_6H_5\,CO\,CH_2(^-)$ formed in the first step accepts a proton more quickly on oxygen than on carbon, and the polarographically inactive enol $C_6H_5\,C\,(OH)=CH_2$ is thus the predominating form in the first reduction step.

This enol is transformed either by an acid-catalysed reaction into the protonized form (2·1), when an increase of the one-electron wave with decreasing *p*H is observed; or by a base-catalysed reaction (2·1′), with subsequent protonation of the ketone formed. The rates of ketone formation and protonation being comparable, the resulting current first increases and then decreases with increase of *p*H (Fig. 2*b*). The decreasing part of this dependence, limited by the rate of protonation, is identical with that found for acetophenone. Finally, the unprotonized keto form due to reaction catalysed by a base can be reduced in a two-electron step (2·3) at more negative potentials.

In accordance with this supposition the dependence on *p*H of the more negative wave is little affected by

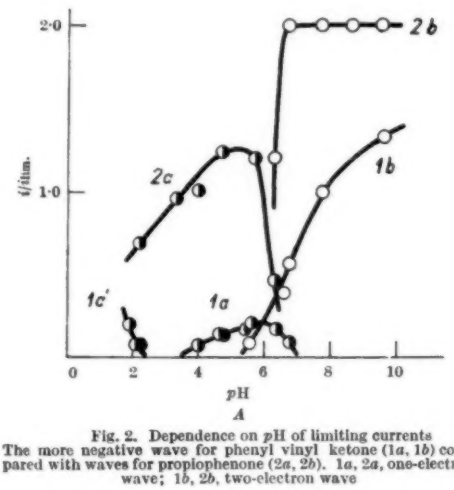


Fig. 2. Dependence on *p*H of limiting currents
The more negative wave for phenyl vinyl ketone (1*a*, 1*b*) compared with waves for propiophenone (2*a*, 2*b*). 1*a*, 2*a*, one-electron wave; 1*b*, 2*b*, two-electron wave

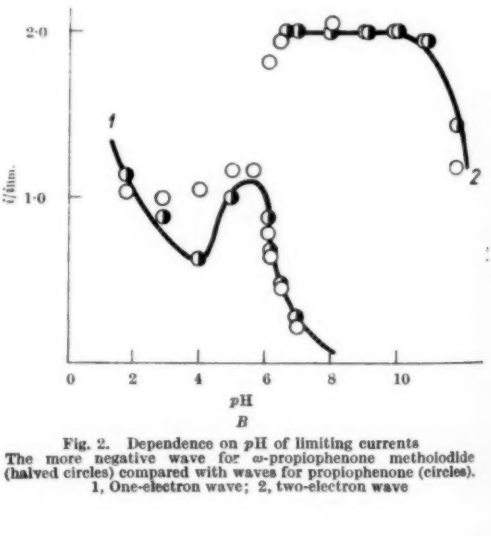


Fig. 2. Dependence on *p*H of limiting currents
The more negative wave for ω-propiophenone methoiodide (halved circles) compared with waves for propiophenone (circles). 1, One-electron wave; 2, two-electron wave

the nature of X in $C_6H_5\,CO\,CH_2$—X, but depends both on substitution in the phenyl ring and on the methylene group[1,7].

The course of the reduction of phenyl vinyl ketone is also consistent with the idea that the reduction of the protonized form leads to inactive $C_6H_5\,CH(OH)CH=CH_2$, while that of the unprotonized form leads to $C_6H_5\,CO\,CH_2\,CH_3$. The increase in height of the wave would be a function of rate of proton transfers only, but this would scarcely explain the increase of the propiophenone wave in acid media.

The effect of keto-enol tautomerism on the polarographic curves of ketones formed at the dropping mercury electrode is being investigated further.

P. Zuman

Polarographic Institute,
Czechoslovak Academy of Science.

J. Michl

Department of Organic Chemistry,
Charles University, Prague.

[1] Zuman, P., and Horák, V., *Collect. Czechoslov. Chem. Comm.*, **26**, 176 (1961).
[2] Michl, J., Thesis, Charles University, Prague (1961).
[3] Calzolari, C., and Furlani, C., *Ann. Chim.* (Roma), **44**, 356 (1954). Holleck, L., and Marsen, H., *Z. Elektrochem.*, **57**, 301 (1953). Kastening, B., and Holleck, L., *ibid.*, **63**, 166 (1959). Elving, P. J., and Leone, J. T., *J. Amer. Chem. Soc.*, **80**, 1021 (1958). Nagata, Y., *Rev. Polarography*, **7**, 12 (1957). Mandell, L., Powers, R. M., and Day, jun., R. A., *J. Amer. Chem. Soc.*, **80**, 5284 (1958).
[4] Ono, S., Takagi, M., and Wasa, T., *Collect. Czechoslov. Chem. Comm.*, **26**, 141 (1961).
[5] Semerano, G., and Chiaini, A., *Gazz. Chim. Ital.*, **66**, 504 (1936). Brdička, R., *Chem. Listy*, **39**, 35 (1945). Vitjajeva, S. I., and Markman, A. L., *Dokl. Akad. Nauk Uzbek, S.S.R.*, No. 8, 33 (1957).
[6] Elving, P. J., and Grodzka, P. G., *Anal. Chem.*, **33**, 2 (1961).
[7] Lund, H., *Acta Chem. Scand.*, **14**, 1927 (1960).

BIOCHEMISTRY

Modification of the Properties of Flour Protein by Thiolated Gelatin

Recently, both Hird's group[1] and Mecham *et al.*[2,3] have suggested that many characteristic properties of dough may be accounted for by postulating that the flour proteins participate in sulphydryl–disulphide interchange reactions. Sokol and Mecham[4] have pointed out, however, that there is as yet no direct evidence that such reactions do occur in dough. Thus further evidence bearing on this attractive hypothesis is desirable.

As a contribution to this question we have examined the effect of directly adding, to dough, protein containing sulphydryl groups. For this purpose we have used gelatin with —SH groups 'built in' by the thiolation procedure of Benesch and Benesch[5], comparing this thiolated protein with normal gelatin which has a negligible content of sulphydryl groups.

The effect on some of the physical characteristics of dough was examined by stretching the doughs on an extensometer, thus obtaining load-extension curves (cf. Halton[6]). Secondly, using these doughs, determinations were made of the content of hydroxyproline in the gluten, as this insoluble protein complex is primarily responsible for the rheological behaviour of dough. Since there is about 14·5 per cent hydroxyproline in the gelatin and none in normal gluten, its presence would indicate an association in the gluten of flour proteins and gelatin.

Unyeasted doughs were made by mixing 28 gm. flour with sodium chloride solution (2·5 per cent). Gelatin, thiolated gelatin or other substances to be added were dissolved in the salt solution immediately before adding it to the flour. After mixing, the doughs were rested for 1 hr. and then stretched on the extensometer. These operations were carried out at 26·6° C. Glutens were then washed from the doughs with warm water and hydrolysed with acid. The neutralized hydrolysates were dissolved in 0·1 *M* citrate buffer, *p*H 2·2, and then passed through a column of 'Zeokarb 225' to separate hydroxyproline from tyrosine, which interferes in the determination. Hydroxyproline was determined, in the appropriate fraction from the column, by the method of Neuman and Logan[7].

The extensometer measurements showed that with freshly prepared samples of thiolated gelatin the extensibility of the dough was increased and the elasticity decreased, as compared with the control doughs. In the control experiments with normal, unthiolated gelatin no significant changes in the load-extension curves were observed. Following Benesch and Benesch, the preparation of thiolated gelatin had been passed through a column of 'Amberlite 400' to remove any *N*-acetyl homocysteine formed during the thiolation reaction. It is to be presumed, therefore, that the observed modification of the physical properties of dough were not due to traces of this compound but to protein sulphydryl.

As additional control experiments we have also made doughs containing mixtures of normal gelatin with respectively cysteine, homocysteine, partially hydrolysed *N*-acetyl homocysteine thiolactone, and determined the hydroxyproline content of the gluten from these doughs. The results in Table 1 show the quantity of gelatin 'bound' per 28 gm. flour, as calculated from the content of hydroxyproline in the various gluten hydrolysates.

Table 1

Additions to dough (28 gm. flour)	Gelatin added to dough (mgm.)	Gelatin 'bound' to gluten (mgm.)
Normal gelatin	156	5
Normal gelatin	156	5
Thiolated gelatin	160	60
	80	38
Gelatin and homocysteine	156	7
Gelatin and cysteine	156	6
Gelatin and partially hydrolysed *N*-acetyl homocysteine thiolactone	160	6
	160	8

These results show that while a significant proportion of the thiolated gelatin becomes firmly associated with the gluten, this degree of binding is not observed with gelatin or with mixtures of gelatin and —SH compounds of low molecular weight. The evidence at present is insufficient to decide whether this is due to interchange reactions leading to the formation of mixed flour protein–gelatin disulphides or whether it is due to the association of the thiolated gelatin by other mechanisms, for example, by hydrogen bonding, or formation of insoluble disulphide gelatin. Experiments are in progress which it is hoped will provide further evidence on this question.

E. E. McDermott
J. Pace

Research Association of British Flour-Millers,
Cereals Research Station,
Old London Road,
St. Albans, Herts.

[1] Frater, R., Hird, F. J. R., Moss, H. J., and Yates, J. R., *Nature*, **186**, 451 (1960).
[2] Mecham, D. K., *Cereal Chem.*, **36**, 134 (1959).
[3] Sokol, H. A., Mecham, D. K., and Pence, J. W., *Cereal Chem.*, **37**, 739 (1960).
[4] Sokol, H. A., and Mecham, D. K., *Bakers' Digest*, **34**, 24 (1960).
[5] Benesch, R., and Benesch, R. E., *Proc. U.S. Nat. Acad. Sci.*, **44**, 848 (1958).
[6] Halton, P., *Monograph No. 6* (Soc. Chem. Indust., London, 1959).
[7] Neuman, R. E., and Logan, M. A., *J. Biol. Chem.*, **184**, 299 (1950).

Separation of Low Molecular Weight Peptides from Amino-Acids on 'DEAE-Sephadex'

Methods are now available for the separation of amino-acids from peptides, with a molecular weight greater than 1,000, by molecular sieving on cross-linked dextran gels[1,2]. However, the separation of peptides of lower molecular weight from all their constituent amino-acids is often difficult because of their similar physical properties. Recently I described a method for the separation of neutral amino-acids from neutral peptides of low molecular weight on the anion exchanger 'Dowex-1' with ammonium acetate buffer *p*H 8·65 (ref. 3). At this *p*H the monoaminomonocarboxylic acids, which all have *pK* values for the amino group greater than 9·0, are predominantly in the neutral form, whereas many peptides of these amino-acids have *pK* values less than 8·3 and thus are predominantly in the anionic form (*pK* values from Cohn and Edsall[4]). This method had the disadvantages that the aromatic amino-acids were strongly adsorbed on the resin, and that traces of amino-acids contaminated the peptide fraction.

An improved method is now described using the anion exchanger 'DEAE-Sephadex', with collidine acetate buffer and a *p*H gradient elution. Rudloff and Braunitzer[5] used this buffer and 'Dowex-1' resin for the fractionation of peptides from a partial hydrolysate of hæmoglobin. This buffer system has several advantages over the ammonium acetate solution used in the earlier method. It gives better control of *p*H, is more volatile, and does not interfere with the estimation of amino-acids and peptides by the ninhydrin reagent[6]. By using a *p*H gradient elution it is possible to separate neutral peptides from acidic amino-acids. Mixtures of pyridine and acetic acid have been recommended as solvents for separation by molecular sieving on 'Sephadex' and 'DEAE-Sephadex' (ref. 2).

There are also advantages in using the diethyl-amino-ethylether (DEAE) derivative of cross-linked dextran gel rather than the quaternary ammonium derivative of polystyrene. DEAE exchangers have a reasonably high capacity in the *p*H 8 region[7]. With 'DEAE-Sephadex' the functional groups are mainly inside the gel grains, and thus when they are charged, ions of opposite charge will be repelled from the grains. Porath and Lindner[2] showed that aromatic compounds, which are strongly adsorbed on polystyrene resins and dextran gels, can be effectively fractionated on 'DEAE-Sephadex', since intimate contact with the strong adsorption centres is avoided. Hence, a separation of aromatic amino-acids from peptides should be possible on this material.

To illustrate the use of this method, a mixture of a typical tripeptide, DL-leucylglycylglycine, and twelve common L-amino-acids was fractionated and the result is shown in Fig. 1. 'DEAE-Sephadex' *A*25 (Pharmacia, Uppsala, Sweden, Lot No. *To*7871*M*; particle size 100–270 mesh) was cycled with 0·5 *M* hydrochloric acid and 0·5 *M* sodium hydroxide and converted to the acetate form with 0·5 *M* acetic acid. A column 20 cm. × 1 cm. diameter was thoroughly equilibrated with 0·1 *M* collidine (2,4,6,-trimethyl-pyridine) acetate buffer *p*H 8·55, prepared from water free from carbon dioxide. The amino-acids were each approximately 2·5 m*M* and the peptide 5·0 m*M* in the buffer with the *p*H adjusted to 8·45.

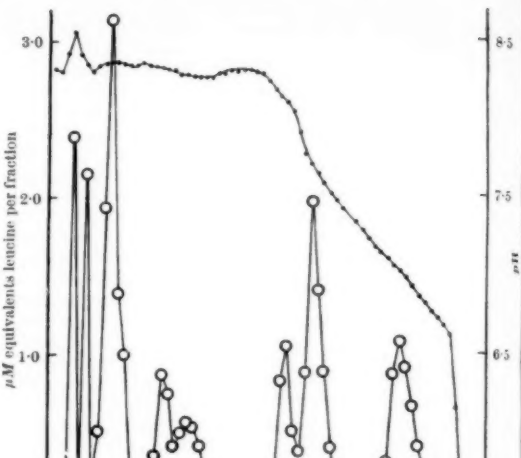


Fig. 1. Separation of DL-leucylglycylglycine from L-amino-acids on a column of 'DEAE-Sephadex' *A*25 (for details see text). ○, total ninhydrin colour of fraction expressed as μ*M* equivalents of leucine; ●, *p*H of fraction; Peak 1, lysine; 2, proline (as μ*M* proline); 3, alanine, glycine, valine, leucine; 4, serine; 5, phenyl-alanine; 6, tyrosine; 7, leucylglycylglycine, cystine; 8, glutamic acid, aspartic acid

Cystine, which would be eluted with the peptide, was first partially oxidized to cysteic acid by treating 1·0 ml. of the mixture with 0·05 ml. 30 per cent hydrogen peroxide and evaporating to dryness, *in vacuo*, in less than 10 min. at room temperature. The mixture was transferred to the column with 1·5 ml. buffer. A total of 20 ml. of buffer was placed between the top of the column and the mixing chamber, which contained 150 ml. buffer. Elution was started and a gradient in *p*H established using 0·1 *M* acetic acid until fraction No. 45, when 1·0 *M* acetic acid was used. Fractions of 2·5 ml. volume were collected at a rate of 11/hr. The temperature was 30° C. (room temp.). From each fraction 0·5 ml. was analysed by the quantitative ninhydrin reagent[6], and the results expressed as leucine equivalents (μmoles per fraction). The *p*H of each fraction was determined (some absorption of carbon dioxide occurred in the more alkaline fractions while they were in the collector, causing a slightly low value for the *p*H). The amino-acid composition was determined by paper chromatography.

The amino-acids were eluted from the column in the order expected from the *pK* values of their amino groups[4]. The aromatic amino-acids were adsorbed to some extent but not so strongly as on the 'Dowex-1' resin. Only fraction No. 40 contained both tyrosine and leucylglycylglycine. Their separation would be improved by re-running and taking smaller fractions. As the *p*H value of the eluant fell below 7·9 the peptide began to emerge. Cystine, because of its slightly acidic character, emerged with the peptide. It was possible to remove only 46 per cent of the cystine by the oxidation procedure used. More vigorous oxidation may damage other amino-acids[8]. However, cystine can be easily removed from most peptides by partition chromatography. When some other neutral peptides

DL-alanylglycine, DL-alanyl-DL-alanine, DL-alanyl-DL-methionine, DL-alanylglycylglycine, glycylylglycine, diglycylglycine, glycl-L-leucine and glycyl-L-tryptophan) were chromatographed on this system, they also emerged when the *p*H value of the eluant fell below 8·0. No difficulty was experienced with the aromatic peptide glycylphenylalanine.

With the earlier method[3] carbon dioxide was found to interfere with the separation, and the formation of carbamino-acids was suggested to explain the presence of amino-acids in the peptide fraction. However, using 'DEAE-Sephadex' it was possible to separate leucine from glycylglycine with 0·1 *M* collidine carbonate buffer *p*H 8·5. This matter is being investigated in greater detail. Recently the separation of amino-acids and peptides on DEAE-cellulose with carbonic acid was reported[8].

I wish to thank Prof. T. F. Dixon for his criticism of the manuscript, Dr. R. L. M. Synge for his interest and encouragement of this work, and S. Kanason for technical assistance.

P. R. CARNEGIE

Department of Biochemistry,
University of Malaya,
Singapore 3.

[1] Porath, J., *Biochim. Biophys. Acta*, **39**, 193 (1960).
[2] Porath, J., and Lindner, E. B., *Nature*, **191**, 69 (1961).
[3] Carnegie, P. R., *Biochem. J.*, **78**, 687 (1961).
[4] Cohn, E. J., and Edsall, J. T., *Proteins, Amino Acids and Peptides*, 84 (Reinhold Publishing Corp., New York, 1943).
[5] Rudloff, V., and Braunitzer, G., *Hoppe-Seylers Z. physiol. Chem.*, **323**, 129 (1961).
[6] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **211**, 907 (1954).
[7] Jakubovic, A. O., and Knight C., in *Chromatographic and Electrophoretic Techniques*, 569 (Heinemann Ltd., London, 1960).
[8] van Halteren, M. B., *Nature*, **168**, 1090 (1951).
[9] Yanari, S., Volini, M., and Mitz, M. A., *Biochim. Biophys. Acta*, **45**, 595 (1960).

Demonstration and Some Properties of Peroxidase in Pig Thyroid Gland

ALTHOUGH it has been postulated by various authors[1,2] that the thyroid contains a peroxidase which plays an essential part in the synthesis of organically bound iodine, the available data do not seem to be sufficient to prove its presence. This communication submits distinct evidence for the presence of peroxidase in pig thyroid tissue, especially in its microsomes.

In this work an assay[3] employing guaiacol instead of iodide as a hydrogen donor was used, since this method is not only sensitive and convenient to carry out but also makes it possible to obtain the initial rate of reaction, which is difficult to measure using iodide as a hydrogen donor. Peroxidase activity in the preparation was expressed in terms of specific and total activity (see Table 1).

It was found by this assay method that thyroid homogenate always revealed fairly strong peroxidase activity although the activity varied considerably from preparation to preparation. The activity in the homogenate disappeared entirely after boiling for 2 min.

The distribution of peroxidase activity in the subcellular fractions is given in Table 1, which shows that both specific and total activity are greatest in the microsomal fraction. Appreciable activity was also found in the nuclear and mitochondrial fractions, but this is thought to be attributable to unbroken cells and concomitant microsomes, respectively. The activity due to contaminating hæmoglobin in the particulates was estimated to be negligibly small on the basis of the measurement of the amount of hæmoglobin

Table 1. INTRACELLULAR LOCALIZATION OF PEROXIDASE IN PIG THYROID

Pig thyroid slices were washed repeatedly with 0·9 per cent sodium chloride solution, blotted with filter paper, and a 10 per cent tissue suspension was homogenized in a 0·25 *M* sucrose solution using a Waring blender. Sediments obtained by differential centrifugation were dispersed uniformly in phosphate buffer, *p*H 7·0. The enzyme assay system contained: 1·0 ml. of 0·1 *M* phosphate buffer, *p*H 7·0; 1·0 ml. of 0·1 *M* guaiacol; an adequate amount of the tissue fraction; 0·027 ml. of 0·03 *M* hydrogen peroxide; and water to a final volume of 3·03 ml. The over-all reaction was followed by a Beckman spectrophotometer at 20° C. by measuring the absorbance at 470 mμ (ref. 3). One unit of peroxidase activity is defined as an increase in absorbance of 1·0 per sec. under the conditions specified above. Specific activity refers to the number of units per mgm. of nitrogen

Fraction	Preparation	Specific activity (milli-units per mgm. nitrogen)	Total activity (milli-units per gm. wet tissue)
Homogenate	*a*	12·5	250
	b	9·5	102
Sediment 700*g*, 10 min. (nuclei)	*a*	19·0	24·0
	b	9·5	31·0
Sediment 5,000*g*, 10 min. (mitochondria)	*a*	16·3	12·1
	b	23·6	11·3
Sediment 80,000*g*, 30 min. (microsomes)	*a*	77·0	58·5
	b	68·1	63·0
Supernatant 80,000*g*, 30 min.	*a*	2·2	17·5
	b	0·9	6·1

a refers to experiments that started from pooled glands of seven pigs; *b* from ten pigs.

contained in the preparations and of the peroxidative activity of crystalline horse hæmoglobin under the same conditions.

The microsomal peroxidase was found to have the following properties: (1) The Lineweaver–Burk plot at a constant concentration of guaiacol (33 m*M*) was linear in the range of the concentration of hydrogen peroxide, 0·3–0·03 m*M*. Beyond a concentration of hydrogen peroxide of 0·4 m*M*, distinct inhibition was observed, increasing with the increase in the hydrogen peroxide concentration. The Lineweaver–Burk plot at a concentration of hydrogen peroxide of 0·27 m*M* was linear in the range of the concentration of guaiacol studied (64–0·9 m*M*). (2) The *p*H optimum was about 8·6 under the conditions of the standard assay system except in the case of *p*H. (3) The enzyme was inhibited by potassium cyanide, sodium azide, and sodium fluoride, in such a way that the percentage inhibition versus the logarithm of the concentration of each reagent represented a sigmoid curve of the first order. The concentrations for 50 per cent inhibition were $2{\cdot}2 \times 10^{-6}$ *M*, $1{\cdot}3 \times 10^{-5}$ *M* and $5{\cdot}8 \times 10^{-3}$ *M*, respectively. The inhibition by cyanide was caused, on the basis of kinetic study, by the competition with hydrogen peroxide for the enzyme. These facts suggest that the enzyme is probably a hæmoprotein, as are other known peroxidases. (4) The enzyme was completely inhibited by thiouracil and 3-amino-1,2,4-triazole at a concentration of 2 m*M*, whereas it only partially inhibited by the same concentration of potassium perchlorate and potassium thiocyanate.

It was found that the microsomal peroxidase was rendered soluble by treatment with 1 per cent digitonin for 40 min. at 2° C. This enzyme was further purified about two-fold by salting-out with ammonium sulphate between 40 and 60 per cent saturation. The purified enzyme revealed practically the same properties as the one bound in the microsomes.

The partially purified enzyme preparation was able to catalyse, in the presence of enzymically generated hydrogen peroxide, the incorporation of substrate amount of iodide into thyroglobulin as efficiently as

thyroid particulate fraction did[4]. It was also possible (unpublished observations of Dr. M. Suzuki of this Institute) for the enzyme preparation to catalyse the iodination reaction of tyrosine under the experimental conditions of Alexander[2]. These results seem to show that the thyroid peroxidase dealt with in this communication catalyses the peroxidation of iodide and probably plays an important part in the hormone synthesis in thyroid tissue.

We thank Dr. M. Suzuki of this Institute for carrying out one of the experiments.

Toichiro Hosoya
Nobuo Ui

Institute of Endocrinology,
Gunma University,
Maebashi, Japan.

[1] Astwood, E. B., in *The Thyroid* (Brookhaven Symposia in Biology, No. 7), 61 (Brookhaven National Laboratory, New York, 1954).
[2] Alexander, N. M., *J. Biol. Chem.*, **234**, 1530 (1959).
[3] Hosoya, T., *J. Biochem. (Japan)*, **47**, 794 (1960).
[4] Kondo, Y., and Ui, N., *Biochim. Biophys. Acta*, **48**, 415 (1961).

Transaminase Activity in Arterial Tissue of the Rabbit

Rinehart and Greenberg[1,2] observed a marked thickening of the intima and deposition of lipid in pyridoxine-deficient monkeys. Though the role of pyridoxine as an essential co-factor in fat and protein metabolism has been well established, the precise mechanism of atherogenesis in pyridoxine deficiency has not been elucidated. Goswami and Sadhu[3] have adduced evidence tending to show that the hypercholesterolæmia of pyridoxine deficiency is not related to impairment of formation of arachidonic acid. The possibility that atherogenesis may be a consequence of defective formation and maintenance of collagen and elastic tissue brought about by decreased transaminase activity therefore gains added importance. However, while the transaminase activities of several tissues, both under normal and pathological conditions, have been examined, very little work has been reported on the transaminase activity of arterial tissue. The present communication reports the demonstration of significant transaminase activity in arterial tissue.

Adult male rabbits weighing approximately 1 kgm. were killed, and different segments of arterial tissue dissected out, homogenized in a Potter–Elvehjem homogenizer at 0° C. with disodium phosphate (0·1 *M*) buffer of *p*H 7·4, and centrifuged at 2,500 r.p.m. for 10 min. in the cold. The supernatant was examined for transaminase activity. Portions were incubated with different L-amino-acids (glutamate, aspartate and alanine) as amino donor and α-keto acids (α-ketoglutarate, oxaloacetate and pyruvate) as amino acceptor. The incubation mixture for the assay of enzyme activities consisted of 1·0 ml. of crude extract (0·3 mgm. nitrogen/ml.), 0·2 ml. of 0·1 *M* amino donor and 0·2 ml. of 0·2 *M* amino acceptor. The samples were incubated for 1 hr. at 37° C. The protein was precipitated by boiling the samples for 10 min., cooling and then adding acetone to 5 ml. volume. The samples were centrifuged and 0·04 ml. of the supernatant fluid was taken for paper chromatographic determination of amino-acid in the reaction product (Table 1).

High transaminase activity was noticed with aspartate + α-ketoglutarate and with glutamate + oxaloacetate. No transaminase activities were noticed with glutamate + pyruvate, aspartate + pyruvate, alanine + α-ketoglutarate or alanine + oxaloacetate.

Table 1. Transamination of Different L-Amino-acids with α-Keto Acids

Amino donor	Amino acceptor	Amino-acid formed
Aspartate	α-Ketoglutarate	Glutamate
Alanine	α-Ketoglutarate	Nil
Glutamate	Oxaloacetate	Aspartate
Aspartate	Oxaloacetate	Nil
Aspartate	Pyruvate	Nil
Glutamate	Pyruvate	Nil

The variation in transaminase activity within the *p*H range 4·0–10·0 was examined using aspartate and α-ketoglutarate. The optimum *p*H was found to be between 7·0 and 8·0; maximum transamination of the keto-acid was observed after 30 min. of incubation, with no further increase thereafter. Ageing of the extract at room temperature (30° C.) for 4–6 hr. or in the refrigerator for 4–5 days did not lower the transaminase activities appreciably.

The transaminase activity was found to be inhibited after pre-incubation of the extract (at 37°) with *p*-chloromercuric benzoate (10^{-4} *M*), mercuric chloride (5×10^{-5} *M*) and silver nitrate (5×10^{-5} *M*). But when incubated with cysteine, *p*-chloromercuric benzoate could not inhibit the transaminase activity. Enzyme activity was unaffected by pre-incubation with deoxypyridoxine (10^{-4} *M*), potassium cyanide (10^{-4} *M*), hydroxylamine hydrochloride (10^{-4} *M*), 2,4-dinitrophenyl hydrazine (10^{-4} *M*), 8·0 oxiquinoline (10^{-4} *M*) and penicillamine (10^{-4} *M*).

Detailed work dealing with the co-factor requirements, purification, kinetics and other properties of the transaminase system in normal and atherosclerotic arterial tissues will be reported elsewhere.

I am grateful to Dr. J. C. Ray, director of this Institute, and to Dr. A. Narayanaswami for their interest in this work.

Dhurjati Prasad Chattopadhyay
Indian Institute for Biochemistry
and Experimental Medicine,
P–27 Prinsep Street,
Calcutta, 13.

[1] Rinehart, J. F., and Greenberg, L. D., *Amer. J. Path.*, **25**, 481 (1949).
[2] Rinehart, J. F., and Greenberg, L. D., *Amer. Med. Assoc. Arch. Path.*, **51**, 12 (1951).
[3] Goswami, A., and Sadhu, D. P., *Biochem. J.*, **78**, 732 (1961).

Interaction of ^{14}C-Œstrone with Tryptophan in the Presence of Peroxidase

In 1955, Stotz *et al.*[1] discovered an œstrogen-sensitive peroxidase in rat uterus and later these workers[2] found that those tissues which had a high rate of cell renewal also had high peroxidase activity. This enzyme may therefore play a significant part in cell proliferation and growth, and it was thought of interest to investigate its effect on œstrogens, using initially the very stable and more readily available horseradish enzyme. In preliminary experiments[3] it has already been shown that œstrone and other phenolic œstrogens could interact with protein and certain amino-acids when incubated with peroxidase under aerobic conditions, and the present communication deals with this reaction involving tryptophan.

(16–^{14}C)œstrone (4,000 c.p.m. in 10 μgm.) dissolved in 5 ml. phosphate buffer *p*H 7·4 was incubated under oxygen with tryptophan (3×10^{-3} *M*), manganese chloride (10^{-5} *M*) and horseradish peroxidase (10 μgm.), for 5 hr. at 37° C. with constant shaking, and the amount of radioactivity remaining in the aqueous medium after extraction with ether was determined. This was repeated under various experimental conditions. In addition, each ethereal fraction was examined for œstrone or its metabolites by chromato-

Table 1. PERCENTAGE CONVERSION OF ^{14}C-ŒSTRONE TO WATER-SOLUBLE ETHER-INSOLUBLE PRODUCTS BY PEROXIDASE IN THE PRESENCE OF TRYPTOPHAN UNDER VARIOUS EXPERIMENTAL CONDITIONS

Experimental conditions	Percentage of added radioactivity remaining in aqueous medium after extraction with ether
Normal incubation *	61·5
	68·5
$MnCl_2$ omitted	4·8
	5·5
Tryptophan omitted	1·1
	1·0
Peroxidase solution boiled	1·6
	1·2
Anaerobic incubation	0·8
Incubated with KCN (10^{-3} *M*)	0·5
Incubated with sodium azide (10^{-3} *M*)	14·5
Incubated with sodium iodoacetate (10^{-3} *M*)	57·3
Incubated with catalase (100 μgm.)	1·1
Kynurenine substituted for tryptophan	5·5
$MnCl_2$ omitted and incubated with H_2O_2 (2×10^{-5} *M*)	14·2
$MnCl_2$ omitted, kynurenine substituted for tryptophan and incubated with H_2O_2 (2×10^{-5} *M*)	13·8
Tryptophan added after incubation and solution immediately extracted with ether	2·2
^{14}C-Œstrone added after incubation and solution immediately extracted with ether	2·0

* Incubation mixture: peroxidase, 10 μgm.; œstrone, 10 μgm.; $MnCl_2$, 10^{-4} *M*; tryptophan, 3×10^{-3} *M*; in 5 ml.

graphy[4] followed by autoradiography[5]. The anaerobic incubation was carried out in an evacuated Thunberg tube.

The results in Table 1 show that horseradish peroxidase, in the presence of tryptophan, is able to convert œstrone to water-soluble ether-insoluble metabolites in high-percentage yields. No such products were formed in the absence of this amino-acid and only unchanged œstrone could be detected in the ethereal fraction, showing that tryptophan was directly involved in the reaction and was not merely acting as a carrier. No water-soluble compounds were formed when tryptophan or œstrone was added only after incubation and the solutions immediately extracted with ether, and there is evidence that the tryptophan molecule was altered during the reaction. This was indicated by the yellow colour of the solution after incubation and the change in ultra-violet spectrum, and was confirmed by chromatography.

The reaction was dependent on manganese ions and was abolished under anaerobic conditions or on boiling the peroxidase solution. Cyanide and azide, which are known to affect iron-containing enzymes, were also found to be inhibitory, but iodoacetate, which combines with sulphydryl groups, had no effect.

The marked inhibition by catalase suggests that hydrogen peroxide is involved in the reaction and although added hydrogen peroxide in the absence of manganese was not very effective, even at concentrations above 2×10^{-5} *M*, the results agree with the findings of Knox and Mehler[6], who showed that only slowly generated peroxide could be utilized efficiently for the breakdown of tryptophan by liver peroxidase-oxidase. Kynurenine, an oxidation product of tryptophan, which is not known to generate hydrogen peroxide with peroxidase, gave only very low results, but with added peroxide the yield of water-soluble products was as high as with the parent amino-acid.

These results suggest that horseradish peroxidase, acting as an aerobic oxidase, can utilize tryptophan to generate hydrogen peroxide and that afterwards, behaving like a true peroxidase, it converts œstrone to metabolites which interact with this amino-acid or its oxidation products. However, the final proof for this hypothesis must await the isolation and identification of the water-soluble complexes and it is also hoped to determine their physiological activity.

We wish to thank Prof. R. L. Noble and the National Cancer Institute of Canada for their support of this work.

P. H. JELLINCK
LOUISE IRWIN

Cancer Research Centre,
University of British Columbia,
Vancouver 8, Canada.

[1] Lucas, F. V., Neufeld, H. A., Utterback, J. C., Martin, A. P., and Stotz, E., *J. Biol. Chem.*, **214**, 775 (1955).
[2] Neufeld, H. A., Levay, A. N., Lucas, F. V., Martin, A. P., and Stotz, E., *J. Biol. Chem.*, **233**, 209 (1958).
[3] Jellinck, P. H., and Irwin, L., *Biochim. Biophys. Acta* (in the press).
[4] Dao, T. L., *Endocrinol.*, **61**, 242 (1957).
[5] Jellinck, P. H., *Biochem. J.*, **71**, 665 (1959).
[6] Knox, W. E., and Mehler, A. H., *J. Biol. Chem.*, **187**, 419 (1950).

Auxin Activity in Ether-insoluble Fractions of Extracts of Rhubarb Petioles

DURING the past four years efforts have been made in this laboratory to evolve methods suitable for the purification of coleoptile growth promoters known to be present in water-soluble, ether-insoluble fractions of plant extracts. In previous work[1] ethanolic extracts from which ether-soluble compounds had been removed were chromatographed directly. In the present work large quantities of elongating rhubarb petioles were extracted, and it was necessary to purify these 'final ethanol extracts' further. Two of the most promising procedures are described here.

(1) *Alumina column purification.* Very clean preparations are obtained when the 'final ethanol extract' is run through an alumina column. It has not been possible to specify the exact conditions of experiment necessary to give an active extract. Fig. 1 shows some of the properties of an active preparation. The general trend of the results has been confirmed in independent experiments, and is similar in many ways to other work on so-called 'interconvertible' auxins[1,2]. Four identical chromatograms were prepared; one was assayed directly (*a*), and selected zones on the others were eluted in ethanol and then re-chromatographed (*b*–*e*). Since the activity is highly labile, distilled water was chosen as the chromatographic solvent, because of its neutrality and speed of running. Relative to the original chromatogram, the eluates show greater activity, except in the case of the R_F 0·55–0·7 eluate (*c*), which lies between two sources of promoter(s) on the original strip. Elution without re-chromatography does not give a comparable release of activity. Thus, when segments of a chromatogram are placed in separate vials and treated with ethanol for 2 hr. (as in the standard elution procedure), then the ethanol evaporated off and bioassay medium added, there is no enhancement (*f*). If the whole chromatogram is eluted and re-chromatographed, there is enhancement (*e*). It may be suggested that losses during manipulation lead to an increase in promotion by diluting the original quantities to less than supra-optimal concentrations. Alternatively, release of promoter(s) from precursor(s) may be involved.

(2) *Tert.-butanol chromatography.* Active preparations quite distinct from those described above can be obtained consistently by applying the 'final ethanol extract' to Whatman No. 3 paper as a strip and chromatographing at 30°–35° C. using *tert.*-butanol as solvent. Much anthocyanin and other material is retained in the R_F 0–0·5 region, while some stimulators are washed farther down the paper (Fig. 2*a*).

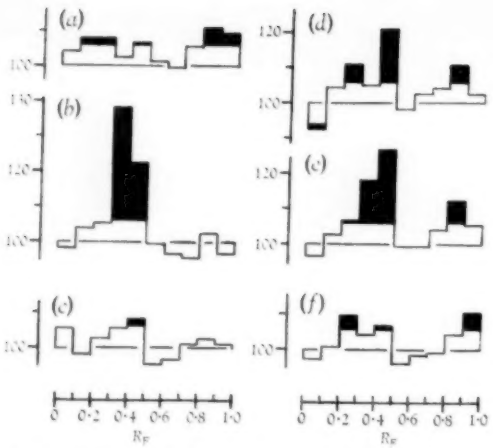


Fig. 1. *Avena* coleoptile straight-growth activity on chromatograms (method as in ref. 1). Vertical scales represent section length as percentage of controls. Responses outside fiducial limits of controls are blacked in. Each chromatogram represents 10 gm. fresh weight of petioles. *a*, Assayed directly; *b*, *c*, *d* and *e*, assayed after zones 0·2–0·5, 0·55–0·7, 0·8–1·0 and 0–1·0 respectively had been eluted and re-chromatographed; *f*, see text. All assays were carried out on the same occasion

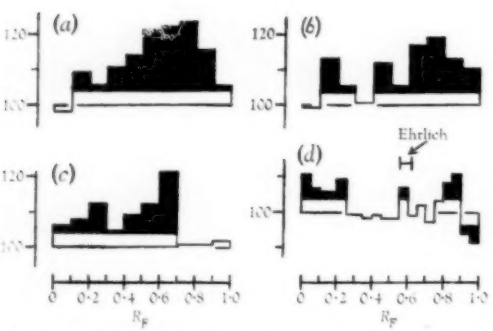


Fig. 2. *a*, *Tert.*-butanol chromatogram of final ethanol extract; *b*, *c* and *d*, are re-chromatographed eluates using distilled water; *b*, isobutanol/methanol/water, 80 : 5 : 15; *c*, and isopropanol/0·880 ammonia/water, 8 : 1 : 1; *d*, as solvents. *d* represents 16 gm., the others 10 gm. fresh weight of petioles

This active zone can then be eluted and re-chromatographed with other solvents on Whatman No. 1 paper. Histograms of activity for three solvents that have been commonly used in investigations of ether-insoluble auxins[1,2] are shown in Fig. 2*b*, *c* and *d*.

Identification of two of the spots resolved by isopropanol / ammonia / water was achieved using an extract of 8 kgm. of petioles:

Spot (i). R_F 0–0·25. Elementary analysis, titrations, ion-exchange chromatography, and infra-red spectrum identified the material as the half ammonium salt of malic acid. Malic acid itself is not a growth stimulator in the assay system used (the medium contains citrate as part of the buffer solution), but the half ammonium salt, and ammonium (as phosphate) are slightly active. This particular spot may therefore be an artefact of ammoniacal chromatography.

Spot (ii). This was located by its Ehrlich reaction (Fig. 2*d*) rather than its weak growth activity. It was eluted and shown to co-chromatograph with $2{\cdot}7 \times 10^{-3}$ μc. aliquots of carbon-14 labelled tryptophan in a series of 21 different one-dimensional solvent systems.

When this *tert.*-butanol method of purification was used checks were made to show that the 'alumina auxins' were absent. There are other indications that the two methods yield quite different substances. For example, the '*tert.*-butanol' group do not show R_F shifts, or release of growth activity when zones of chromatograms run using water as solvent are eluted and re-chromatographed. Again, they are stable, and do not pass through an alumina column.

B. E. S. Gunning

Department of Botany,
Queen's University, Belfast.

[1] Audus, L. J., and Gunning, B. E. S., *Phys. Plant*, **11**, 685 (1958).
[2] Bentley, J. A., *Ann. Rev. Plant Phys.*, **9**, 47 (1958), and *J. Mar. Biol. Assoc. U.K.*, **39**, 433 (1960). Hemberg, T., *Phys. Plant*, **11**, 284 (1958). Lahiri, A. N., and Audus, L. J., *J. Exp. Bot.*, **11**, 341 (1960). Britton, G., and Housley, S., *Phyton*, **16**, 1 (1961).

PHYSIOLOGY

Peroxide-like Substance in Alarmed Mice

While studying the production of an oxidizing agent which could be extracted with butanol (hereinafter called 'apparent peroxide') in X-irradiated mice, we found that some was produced without irradiation, merely by transferring the mouse somewhat roughly to the small irradiation chamber. (Work on irradiated and dummy-irradiated mice will be submitted to the *International Journal of Radiation Biology*.) As this phenomenon may be of physiological interest we have made a few further observations on it.

The apparent peroxide was estimated with anaerobic leuco brilliant cresyl blue, approximately as previously described[1]. The temporal sequence of experimental and control mice was randomized to eliminate secular effects. Table 1 shows that merely lifting mice by their tails with rubber gloves (to avoid possible transfer of peroxide from the hands) produced a statistically significant difference in peroxide between six experimental animals and six controls. Table 2 shows the larger effect of lifting by the scruff of the neck with polythene-covered forceps. Fig. 1 shows that the increase was maximal within 1 min. after lifting with forceps and that it fell to half the maximum in about 97 min.

Table 1

Micromoles of peroxide/gm. of mouse	
Not handled	Lifted by tail
0·13	0·24
0·19	0·28
0·23	0·25
0·24	0·27
0·28	0·25
0·20	0·27
Mean 0·21	0·26

The difference in means would occur by chance about 1 in 50 times and is therefore significant.

Table 2

Micromoles of peroxide/gm. of mouse	
Not handled	Lifted by scruff of neck with forceps
0·23	0·35
0·13	0·39
0·27	0·33
0·25	0·36
0·24	0·37
Mean 0·22	0·36

The difference in means would occur by chance less than 1 in 1,000 times and is therefore significant.

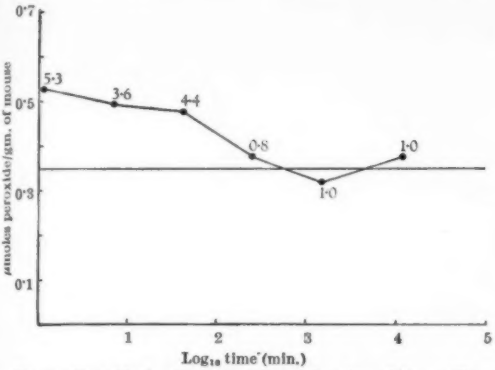


Fig. 1. Peroxide in alarmed mice as a function of time. The figure beside the points are a value of the statistic t between each experimental group (wavy line) and the untreated group (horizontal straight line). The 5 per cent and 1 per cent levels of t are 2·18 and 3·05 respectively. The biological half-life of the peroxide is estimated by interpolation to be 97 min. Each point represents a mean of seven mice. The times were randomized in a Latin square

The identity of the peroxide-like substance or substances concerned is unknown. Adrenochrome, a possible though doubtful metabolite of adrenaline, has the necessary chemical properties; but Table 3 shows no mean difference between mice injected intraperitoneally with adrenaline and with saline, though both increase the apparent peroxide presumably through alarm from the injection.

Table 3

Micromoles of peroxide/gm. of mouse

	Injected with saline	Injected with adrenaline
	0·29	0·38
	0·29	0·34
	0·36	0·25
	0·33	0·22
	0·38	0·44
Mean	0·33	0·33

With adrenocorticotrophic hormone (Table 4) only a small mean difference above saline, statistically insignificant with groups of five mice, was found.

Table 4

Micromoles of peroxide/gm. of mouse

	Injected with saline	Injected with adrenocorticotrophic hormone (0·05 unit/mouse)
	0·25	0·38
	0·30	0·33
	0·25	0·24
	0·35	0·37
	0·24	0·31
Mean	0·28	0·33

The difference in means would occur by chance about 1 in 5 times and is not significant.

It is therefore possible that the present phenomenon depends on some hitherto unknown component of the total response to alarm, involving perhaps autoxidation of unsaturated substances such as linoleic acid to form hydroperoxides.

V. J. Horgan
J. St. L. Philpot

Medical Research Council
Radiobiological Research Unit,
Harwell, Didcot, Berks.

[1] Horgan, V. J., Philpot, J. St. L., Porter, Barbara W., and Roodyn, D. B., *Biochem. J.*, **67**, 551 (1957).

Evidence for Independence of Voltage of the Membrane Conductance of Rabbit Ventricular Fibres

At the present time the available evidence indicates that the prolonged repolarization phase of the cardiac action potential is associated with a drop in the membrane conductance to potassium ions (g_K) which occurs during, or shortly after, the upstroke of the action potential and persists for the major part of the repolarization phase. Noble[1] has put forward a mathematical model which reproduces Purkinje-like action potentials. In this model, g_K is an instantaneous function of the membrane potential and falls when the membrane is depolarized.

We have previously shown[2,3] that the displacement of membrane potential produced by hyperpolarizing current applied during the repolarization phase of rabbit ventricular action potentials bears a linear relationship to the magnitude of the applied current. This finding suggests that the ionic conductance of the membrane does not change with the value of the membrane potential, as it should were it to conform with Noble's model.

The following experiment further affirms the independence of voltage of the ionic conductance of membrane action potentials of rabbit ventricular fibres. The tissue was stimulated at a rate of 3·4 sec.$^{-1}$. Double-barrelled microelectrodes were used together with a current clamp and associated apparatus previously described[4]. Hyperpolarizing current was passed through the cell membrane via one barrel of the electrode, the other barrel recording the resulting potential change. The current was started approximately 50 msec. before the upstroke of every fourth propagated action potential and terminated during the early half of the repolarization phase. A typical result is shown in Fig. 1. A fairly accurate estimate of the hyperpolarization produced at any time is afforded by superimposing the crests of the two action potentials and measuring the potential displacement of the normal action potential and that occurring during flow of current[4].

If the ionic conductances were dependent on voltage then, during the hyperpolarization, they would assume new values, so that, at the break of the current pulse, the subsequent time course of potential

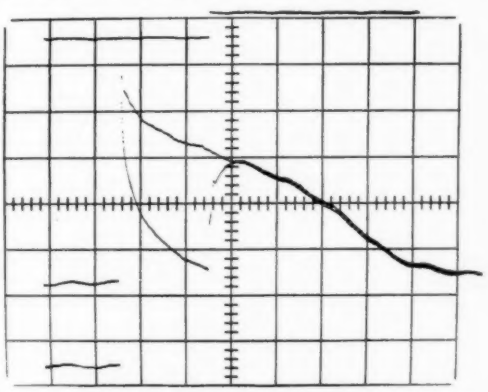


Fig. 1. Top trace: current (hyperpolarizing); calibration, 0·25 μamp. per large division. Lower trace: upper of the two, an action potential recorded before the application of current; lower trace, action potential recorded during current flow. The current pulse was started 50 msec. before the upstroke of the action potential and terminated, as shown, during the repolarization phase. Calibration: 25 mV. and 20 msec. per large division

should differ from that of a normal action potential. The outcome of the experiment is clearly shown in Fig. 1, where the voltage displacement is close to 50 mV. just prior to the break of the current. After the break of the current the membrane potential returns within 10 msec. to a time-course identical to that of the preceding normal action potential. During this 10-msec. interval, a brief voltage spike may occur. Its amplitude depends on the value of the current and the time of break of the current. We believe that this spike is the result of a localized, non-propagated increase in sodium conductance subsequent to 'reactivation' produced by the preceding hyperpolarization. However, even in these instances, the membrane potential returns to its normal time-course after the spike.

These facts are in accord with the hypothesis that a sodium-carrying mechanism dependent on voltage exists in rabbit ventricular fibres, but that the subsequent decrease in g_K, which normally follows the activation of such a sodium-carrying mechanism, is independent of voltage.

This investigation was supported by the Life Insurance and Medical Research Fund of Australia and New Zealand.

Edward A. Johnson
J. Tille

Department of Pharmacology,
University of Sydney.

[1] Noble, D., *Nature*, **188**, 495 (1960).
[2] Johnson, E. A., and Tille, J., *Austral. J. Exp. Biol. and Med. Sci.*, **38**, 509 (1960).
[3] Johnson, E. A., Tille, J., and Wilson, Lesley, *Austral. J. Exp. Biol. and Med. Sci.*, **39**, 179 (1961).
[4] Johnson, E. A., and Tille, J., *J. Gen. Physiol.*, **44**, 443 (1961).

Effect of L-Glutamic Acid on the Weight of Embryonic Chick Tibiotarsi cultivated *in vitro*

In a previous communication[1] the dose-response line relating the final length of embryonic chick tibiotarsi cultivated *in vitro* and the concentration of L-glutamine in the medium was described. Furthermore, it was shown that the presence of 25 mgm. per 100 ml. L-glutamic acid in the medium had no effect on the position of the L-glutamine dose-response line. From this result it was argued that L-glutamic acid does not act as a precursor for L-glutamine in this system. The purpose of the present communication is to present additional results on the wet and dry weights of the same bones. These results were not included in the first communication since their discussion was irrelevant to the point at issue, and could have created confusion.

The experiment was a 2×3 factorial design: 1, 5 and 25 mgm. per ml. L-glutamine and 0 and 25 mgm. per 100 ml. L-glutamic acid were incorporated in all six treatment combinations in the chemically defined medium *BGJb*[2]. The experimental error was reduced by utilizing a balanced incomplete block design for comparing six treatments in blocks of two, thereby exploiting the uniformity of the pair of bones from each embryo. The bones were cultivated, using the floating lens paper technique, at 37° C. for six days in an atmosphere of humidified 5 per cent carbon dioxide in air. The medium was renewed every second day. At the end of the experiment the wet weight and dry weight of each bone were determined on a Cahn electric microbalance using the techniques described elsewhere[3]. The analyses of variance were done after all the data were converted to logarithms.

Table 1. Adjusted Mean Log Wet Weights (μgm.) and Mean Log Dry Weights (μgm.) of Seven-Day Old Embryonic Chick Tibiotarsi after Cultivation for 6 Days on Medium *BGJb*, in the Presence of Various Combinations of L-Glutamine and L-Glutamic Acid. Each Mean is based on Five Observations

L-Glutamic acid (mgm. per cent)	L-Glutamine (mgm. per cent) log wet weight 1	5	25	log dry weight 1	5	25
0	0·032	0·088	0·305	1·932	1·979	2·177
25	0·102	0·230	0·403	2·062	2·104	2·259

Analyses of variance
(Calculated using the methods of Kramer and Bradley, ref. 4)

Source of variation	D.F.	Mean squares log wet weight	log dry weight
Between embryos (unadj.)	14	0·0175	0·0164
Between treatments (adj.)	(5)		
L-glutamic acid	1	0·0481*	0·0566*
L-glutamine:			
Linear	1	0·2480‡	0·1463†
Quadratic	1	0·0105	0·0176
Interaction	2	0·0020	0·0011
Intrablock error	10	0·0055	0·0093

*$0{\cdot}05 > P > 0{\cdot}01$; †$P < 0{\cdot}001$; ‡$0{\cdot}01 > P > 0{\cdot}001$

The adjusted means are shown in Table 1, together with the analyses of variance. As with the results on length[1] there are highly significant linear regressions of log wet weight and log dry weight on the logarithm of the concentration of L-glutamine in the medium. The mean squares for L-glutamic acid are both significant at the $P = 0{\cdot}05$ level. Inspection of the adjusted means shows that the presence of L-glutamic acid has increased the wet and dry weights of the bones at all levels of L-glutamine. Since the interaction mean square is not significant in either analysis of variance we may assume that the L-glutamine dose-response lines for both responses, in the presence and absence of L-glutamic acid, are parallel. The vertical distance between these lines is an estimate of the logarithm of the ratio between the weight of a bone in the presence of L-glutamic acid and the weight of a bone in the absence of L-glutamic acid. These ratios, and their fiducial limits of error ($P = 0{\cdot}05$), have been calculated from the data in Table 1, and are found to be: wet weight: 1·27 (1·06–1·52); dry weight: 1·30 (1·03–1·64). The corresponding ratio for the data on length is 1·03 (0·90–1·18). The difference between the results for length and wet and dry weights is therefore considerable. Further statistical analysis showed that L-glutamic acid and L-glutamine had no significant effects on the dry weight/wet weight ratios of the rudiments.

The results demonstrate that the weights of embryonic chick tibiotarsi are increased by supplementing medium *BGJb* with L-glutamic acid. The increase is 27 per cent in wet weight and 30 per cent in dry weight. Medium *BGJb* contains twelve essential amino-acids and L-glutamine[2]. Although L-glutamic acid can be synthesized by these rudiments from glucose[4] the amount produced in this medium appears to be insufficient to produce a maximal response. In earlier work on the effect of amino-acid deficiencies on the growth in wet weight of chick tibiotarsi[7] it was shown that L-glutamic acid could be omitted from medium 858, which contains 20 essential and non-essential amino-acids, without affecting the response in wet weight. It seems likely, therefore, that when L-glutamic acid is omitted from medium 858, some other non-essential amino-acid can substitute for it. This could be either by its conversion to L-glutamic acid, or through its having an identical physiological activity.

We should not be dismayed that the results on length and the weights of the bones do not agree, since there is increasing evidence[2] that the elongation of the chick tibiotarsus is caused largely by hypertrophy of the diaphyseal chondrocytes, and that mitosis and the secretion of matrix by the chondrocytes play only a minor part. Thus L-glutamic acid probably produces its effects on the weights of the rudiments by stimulating mitosis and/or the secretion of matrix, and has little connexion with the mechanism of hypertrophy. The activity contrasts sharply with the effect of L-glutamine, which seems to be important for elongation as well as growth in weight.

Work on the nutritional requirements of whole animals has shown that L-glutamic acid is not required for adult rats, men, and roosters; it is required, however, for the optimum growth of young chicks, rats and mice, and for egg production by hens[3]. In all these investigations the basal diet contained a minimal number of amino-acids. The results described in this communication on the nutrition of an organ growing *in vitro*, on a chemically defined medium containing a minimal number of amino-acids, are remarkably similar to the results on whole animals.

This investigation was supported in part by U.S. Public Health Service research grant *C*-161–60 from the National Cancer Institute, Public Health Service.

J. D. Biggers

The Wistar Institute,
Philadelphia 4,
Pennsylvania.

[1] Biggers, J. D., *Nature*, **189**, 1016 (1961).
[2] Biggers, J. D., Gwatkin, R. B. L., and Heyner, S., *Exp. Cell Res.* (in the press).
[3] Biggers, J. D., *J. Exp. Zool.*, **145**, 227 (1960).
[4] Kramer, C. Y., and Bradley, E. A., *Biometrics*, **13**, 197 (1957).
[5] Biggers, J. D., and Heyner, S., *J. Exp. Zool.* (in the press).
[6] Webb, M., and Biggers, J. D., *Biochim. Biophys. Acta* (in the press).
[7] Biggers, J. D., Webb., M., Parker, R. C., and Healy, G. M., *Nature*, **180**, 825 (1957).
[8] Biggers, J. D., *Experientia*, **13**, 483 (1957). Biggers, J. D., and Heyner, S., *J. Exp. Zool.* (in the press.)
[9] Leveille, G. A., and Fisher, H., *J. Nutrition*, **69**, 289 (1959).

Effects of Intraventricularly Injected Anti-N. Caudatus Antibody on the Electrical Activity of the Cat Brain

It is well known that anti-tissue antibodies, when injected into an animal of the donor species, may exhibit the selective affinity for the homologous organ and produce certain modifications in its morphological and functional state[1]. The damaging effects of anti-tissue antibodies have been demonstrated in different organs[2]. However, no information could be found in the literature available concerning the physio-pathological events associated with an *in vivo* collision between anti-brain antibodies and the corresponding nervous tissue. Bearing in mind the high complexity of morphological and functional organization of the brain, a series of immuno-electro-physiological experiments has been undertaken to investigate the possible differential reactivity of various cerebral structures when exposed to the action of different brain region anti-sera. The present communication, as a part of these investigations, is a preliminary report concerning only some of the effects of intraventricularly administered anti-n. caudatus antibody on the electrical activity of various parts of the cat brain.

Cats operated under 'Nembutal' anæsthesia were used. Surface and depth, 'Teflon'-insulated silver and stainless steel electrodes (0·005–0·01 in. in diameter), respectively, were implanted into various regions of the brain (frontal cortex, frontal white matter, n. caudatus, hippocampus, hypothalamus, thalamus and brain stem reticular formation). Needle electrodes for the interior of the brain were introduced by means of a Horsley-Clarke stereotaxic instrument. All electrodes were implanted into the left cerebral hemisphere, while the cannula was inserted into the right lateral ventricle, according to the method of Feldberg and Sherwood[3]. Electrical activity was recorded throughout the experiment using a 12-channel Reega-Alvar electroencephalograph. Details regarding recording and stimulation technique have been published elsewhere[4].

The cat n. caudatus was carefully dissected, homogenized, mixed with Freund adjuvant and injected subcutaneously into rabbits (1·0 gm. of fresh tissue per kgm. body-weight). The γ-globulin from the immune rabbit sera was separated by the method of Basset *et al.*[5]. Immune rabbit γ-globulin was investigated by diffusion-gel[6] and complement fixation reaction[7], in which the saline extract of n. caudatus tissue was used as antigen. The reaction between n. caudatus antigen and the corresponding immune rabbit γ-globulin revealed 2–3 precipitin lines in the agar and a titre of 1/128–1/256 in the complement fixation test. Normal rabbit γ-globulin, taken prior to the immunization, did not show any serological activity with antigen prepared from the n. caudatus tissue.

The cats were divided into three groups: the first (experimental group) was treated with immune rabbit γ-globulin; the second (control group) received normal rabbit γ-globulin, and animals from the third (control group) were injected with physiological solution in order to evaluate the possible influence of volume of injected fluid. Two to three injections of 0·3 ml. daily, with an interval of 60 min. between the injections, were administered on four successive days. Total protein content of immune rabbit γ-globulin injected into an animal of the first group was 46·08 mgm., while that of normal γ-globulin administered to an animal of the second group amounted to 60·24 mgm.

Some of the results are illustrated in Fig. 1. In both control groups no essential change in electrical activity of various cerebral structures investigated could be noticed. Records made in the course of normal γ-globulin application, as well as the records taken at intervals within the period of one month following the last injection, were found to be fairly comparable with the activity recorded prior to the γ-globulin administration (Fig. 1*A*). However, in the experimental group of animals, intraventricular application of anti-n. caudatus γ-globulin was followed by pronounced modification in electrical activity. The evolution of electrographic disturbances seemed to pass through three consecutive stages. The first stage was characterized by the appearance of spikes and high-voltage sharp waves localized in the n. caudatus. These transient irritative abnormalities become apparent on the third day of antibody administration, and disappeared two to three days afterwards. The second, also transient stage was characterized by slight general accentuation of background activity and by sporadic appearance of diffuse abnormalities consisting of bursts of 4–6/sec. high-voltage waves, in all leads. Such activity outlasted the disappearance of spikes and sharp waves in n. caudatus for several days. The

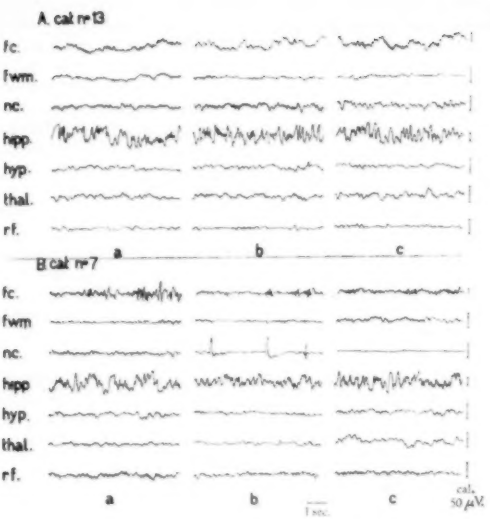


Fig. 1. *A*, Records made (*a*) before administration, (*b*) following the third day of injecting, and (*c*) eight days after the last injection of normal rabbit γ-globulin. *B*, Records taken (*a*) prior to antibody application, (*b*) after the third day of injecting, and (*c*) seven days following the last intraventricular injection of anti-n. caudatus γ-globulin. *fc*, frontal cortex; *fwm*, frontal white matter; *nc*, n. caudatus; *hip*, hippocampus; *hyp*, hypothalamus; *thal*, thalamus; *rf*, brain stem reticular formation; for explanation, see text

third stage was characterized by gradual slowing down, progressive decrease in the amplitude and almost complete disappearance of spontaneous electrical activity of the caudate nucleus within a month after antibody administration (Fig. 1*B*). Such an evolution of electrographic abnormalities was reflected in modification of potentials in n. caudatus evoked by acoustic stimuli, as well as in the change of activity propagated to n. caudatus during seizures induced by electrical stimulation of the hippocampus. It should be emphasized that no change in electrical activity of the caudate nucleus could be observed in animals treated with anti-hippocampus antibody (unpublished experiments).

In a previous paper[7] it was found that lipids from various regions of cat brain lack individual specificity but are all highly organ-specific. That is why, in the present experiments, the saline extract of n. caudatus containing mainly protein components has been used as antigen in preparing the corresponding anti-serum. N. caudatus was chosen because it forms the wall of the lateral ventricle and could be, therefore, easily attacked by the intraventricular route and thus brought into prolonged contact with antibody injected into the cerebrospinal fluid. This, of course, could not be assured adequately if the antibody were administered by a systemic route[8]. Diffuse electrographic abnormalities reported here were also observed following intraventricular administration of some other anti-brain region sera, such as anti-hippocampus, and should be, therefore, considered as non-specific manifestations. However, the initial irritative phenomena, followed by a progressive decrease in electrical activity, strictly confined to n. caudatus, strongly suggest that anti-n. caudatus antibody has definite affinity towards the homologous nervous tissue.

This work has been supported in part by a grant from the Yugoslav Foundation for Scientific Research, contracts No. 490/1 and No. 202/1.

Lj. Mihailović
B. D. Janković

Institute of Pathological Physiology,
Medical School, and Microbiological Institute,
School of Pharmacy,
University of Belgrade.

[1] Pressman, D., and Sherman, B., *J. Immunol.*, **67**, 21 (1951). Bale, W. F., and Spar, L. I., *ibid.*, **73**, 125 (1954). Anigstein, L., McConnell, K. P., Whitney, D. M., Pappas, P., Portman, O. W., and Barnes, W., *Tex. Rep. Biol. Med.*, **12**, 945 (1954).

[2] Metshnikoff, E., *Ann. Inst. Pasteur*, **14**, 369 (1900). Joanović, Dj., in *Allergie*, 707 (Georg Thieme Verlag, Stuttgart, 1957). Masugi, M., *Beitr. Path. Anat.*, **91**, 82 (1933).

[3] Feldberg, W., and Sherwood, S. L., *J. Physiol.*, **120**, 3 *P* (1953).

[4] Mihailović, Lj., and Delgado, J. M. R., *J. Neurophysiol.*, **19**, 21 (1956).

[5] Basset, C. A. L., Campbell, D. H., Evans, V. J., and Earle, W. R., *J. Immunol.*, **78**, 79 (1957).

[6] Ouchterlony, P., *Acta Path. Microbiol. Scand.*, **32**, 231 (1953).

[7] Janković, B. D., Isaković, K., and Mihailović, Lj., *Int. Arch. Allergy*, **17**, 211 (1960).

[8] Stern, L., Kassil, G. N., Lockshina, E. S., Romel, E. L., and Zeitlin, S. M., *C.R. Soc. Biol., Paris*, **99**, 360 (1928).

Effect of Colchicine on Rat Liver Xanthine Oxidase

Colchicine has been shown to inhibit deoxyribonuclease and de-phosphorylation and de-amination of deoxyribonucleotides[1]. The beneficial effect of colchicine in gout is possibly due to the effect of this drug on purine metabolism, since human serum nucleotidase and guinea pig liver xanthine dehydrogenase are inhibited by the alkaloid—the last-named only when given in large doses[2]. The purpose of the present communication is to investigate the effect of colchicine on rat liver xanthine oxidase, and on the purified enzyme.

Male Wistar white rats on a well-balanced diet were used throughout this work. Liver xanthine oxidase was determined from the oxygen uptake in a conventional Warburg apparatus at 37° C., using hypoxanthine as a substrate and air as the gas phase[3]. The xanthine dehydrogenase activity was measured using the anaerobic reduction of the colourless dye triphenyltetrazolium chloride in a Thunberg vacuum tube as suggested by Villela[4]. Pyrophosphate buffer (0·015 *M*), *p*H 8·6, was used in both determinations. The results were expressed in mm.^{3} oxygen/gm. dry weight/60 min. for xanthine oxidase and in μgm. formazan/gm. dry weight/30 min. for xanthine dehydrogenase activity.

Colchicine (0·1 mgm./100 gm. body-weight) was administered by intraperitoneal injection of an alcoholic solution (6·8 per cent) containing 20 mgm.

Table 1. Liver Xanthine Oxidase and Xanthine Dehydrogenase of Rats Injected with Colchicine and with Thiocolchicoside

Conditions	No. of rats		Xanthine oxidase* (mm.^{3} O_2/gm. dry weight in 60 min.)	Xanthine dehydrogenase* (μgm. formazan/gm. dry weight in 30 min.)
	Xanthine oxidase	Xanthine dehydrogenase		
Normal	4	11	1,656 ± 152	227 ± 56
Alcoholic solution (control)	16	15	1,208 ± 328	159·7 ± 45·1
Colchicine (0·1 mgm.)†	13	12	739 ± 231	56·6 ± 22·5
Thio-colchicoside (0·2 mgm.)†	2	3	1,539	250
Thio-colchicoside (0·4 mgm.)†	2	3	1,343	242

* Average values ± S.D.; S.D. = $\sqrt{\Sigma(d^2)/n-1}$.
† Per 100 gm. body-weight.

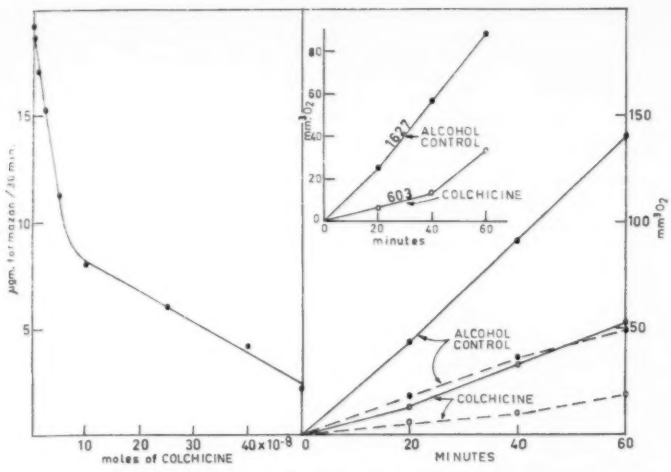


Fig. 1. Rat liver xanthine oxidase and xanthine dehydrogenase inhibition by colchicine. Left, *in vitro* inhibition of purified xanthine dehydrogenase activity. Right, *in vivo* inhibition of liver xanthine oxidase activity. The graph in the upper part represents values of net oxygen uptake in mm.³ oxygen/gm. dry weight/60 min.

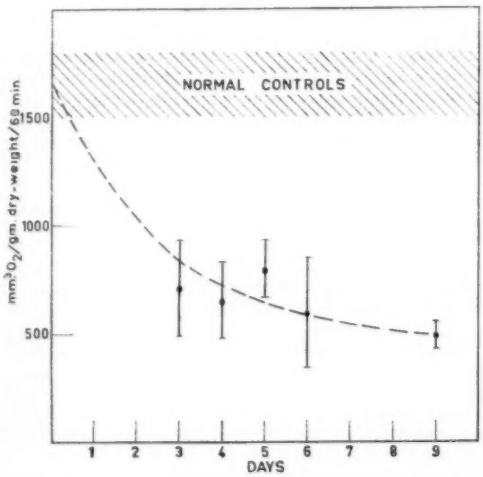


Fig. 2. Rat liver xanthine oxidase inhibition by colchicine in diet. Dotted line of the curve represents the decrease of activity. Abscissa: Time in days in which the rats were fed with colchicine

of colchicine per 100 ml. Blanks were performed by injecting a 6·8 per cent alcoholic solution. Colchicine was administered orally *ad libitum* by adding colchicine (Houdé) to the diet (5 mgm./100 gm.).

For *in vitro* experiments purified liver xanthine oxidase was prepared by solubilization of the enzyme with butanol[5] after the digestion of rat liver homogenate (in 0·2 *M* Na_2HPO_4) with crude trypsin at pH 7·3. After precipitation with ammonium sulphate between 30 and 60 per cent saturation, the enzyme was adsorbed in $Ca_3(PO_4)_2$ and eluted with 0·1 *M* pyrophosphate buffer, *p*H 8·6.

Experiments *in vitro* with 5×10^{-7} mole of colchicine did not show any statistically significant difference in the activities of xanthine oxidase or xanthine dehydrogenase when rat liver homogenate in pyrophosphate buffer was used. However, with purified enzyme, an inhibitory effect was obtained even with 10^{-9} mole of colchicine (Fig. 1, left).

Values for normal rats and controls injected with alcohol as well as rats fed with colchicine are reported in Table 1. A statistically significant inhibitory effect was observed with colchicine for the liver enzyme activity (for xanthine oxidase, $t = 4{\cdot}50$; $P < 1$ per cent; for xanthine dehydrogenase, $t = 7{\cdot}75$; $P < 1$ per cent) (Fig. 1, right). When the drug was administered orally the inhibition was noted in the first days (Fig. 2).

Thio-colchicoside ($C_{32}H_{39}O_{10}NS$), a sulphur-containing analogue of colchicine without antimitotic activity, was used in some other preliminary experiments but appeared to be without any inhibitory effect (Table 1).

The results reported indicated that colchicine has an inhibitory effect on liver xanthine oxidase and xanthine dehydrogenase activities when given to rats orally or in the peritoneum. Although the liver homogenate protects this effect *in vitro*, the purified enzyme is inhibited with small doses of the drug.

This investigation was supported in part by grants (to O. R. A. and E. M.) from the Conselho Nacional de Pesquisas.

Ottilia R. Affonso
Emilio Mitidieri
Gilberto G. Villela

Biochemical Laboratory,
Instituto Oswaldo Cruz,
Rio de Janeiro.

[1] Ebner, H., and Strecker, H., *Experientia*, **6**, 388 (1950).
[2] Keeser, E., *Arch. Exp. Path. Pharm.*, **197**, 187 (1941).
[3] Villela, G. G., and Mitidieri, E., *Nature*, **175**, 208 (1955).
[4] Villela, G. G., *Rev. Bras. Biol.*, **14**, 455 (1954).
[5] Klenow, H., and Emberland, R., *Arch. Biochem. Biophys.*, **58**, 276 (1955).

Effect of Hydrocortisone on Uptake of Iron-59 in Incubated Rat Red Cells *in vitro*

Hydrocortisone added to blood containing reticulocytes in a suitable substrate did not alter the rate of synthesis of hæmoglobin and no evidence was obtained that it influenced the rate of ripening of reticulocytes. This applied to various dosage-levels *in vitro* and to plasma obtained from animals which had been injected with large doses of hydrocortisone. It was shown, however, that preliminary incubation of reticulocytes with hydrocortisone before the addition of iron-59 reduces the amount of iron-59 which enters the reticulocytes.

Reticulocytosis was induced in laboratory rats by intraperitoneal injection of phenylhydrazine hydrochloride. Five days later, blood was removed by cardiac puncture and collected in heparin. Iron-59 uptake was measured by incubating 1-ml. portions of blood with hydrocortisone sodium succinate (supplied by courtesy of the Upjohn Chemical Co.) in physiological saline. About 0·4 μc. of iron-59 as ferric chloride was added at various times, the cells incubated for a further 2 hr., washed 10 times with physiological saline and assayed with an EKCO scintillation counter.

In all instances the uptake of iron-59 in the presence of hydrocortisone was compared with the uptake in the presence of sodium succinate, because it has been shown that preliminary incubation itself reduces uptake. It was found that the amount of iron-59 entering the reticulocytes was not altered by preliminary incubation for up to 5 hr. in the presence of hydrocortisone at concentrations between 0·0083 and 0·500 mgm./ml. of blood. However, an increase in the concentration to 1·0, 1·5 and 2·0 mgm./ml. resulted in a reduction of uptake of iron-59 which was proportional to the concentration of hydrocortisone.

This effect of hydrocortisone is shown in the following experiment in which reticulocytes were incubated for different times with varying concentrations of hydrocortisone. Blood samples of 1 ml. were incubated for 2 and 5 hr. in the presence of 0·5 or 1·0 mgm. hydrocortisone. Iron-59 was added and the uptake observed at 2, 4 and 6 hr. Hydrocortisone did not influence the amount of iron-59 in the cells with only 2 hr. incubation. It was also without effect, compared with the controls, when the cells were incubated with 0·5 mgm./ml. for 5 hr. There was, however, a marked decrease in the total uptake of iron-59 after 5 hr. incubation with 1·0 mgm. hydrocortisone and this effect was still further increased when the cells were suspended in saline instead of their own plasma.

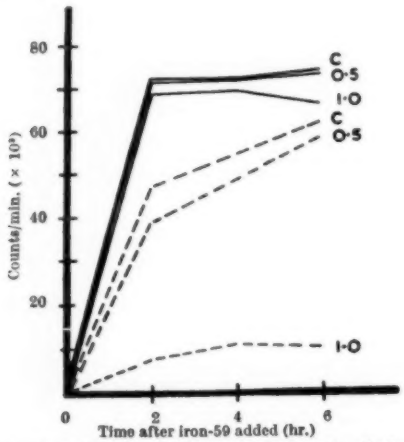


Fig. 1. Uptake of iron-59 in pre-incubated rat reticulocytes. ——, 2 hr. pre-incubation; – – – –, 5 hr. pre-incubation

Warburg experiments on 2-ml. blood samples showed a slight decrease in oxidative respiration in the presence of hydrocortisone sodium succinate, but a similar decrease was found in sodium succinate. Incubation with 1·0 mgm./ml. hydrocortisone resulted in a marked increase in the rate of outward movement of potassium, but incubation with 0·5 mgm./ml. hydrocortisone did not produce a significant increase.

Hydrocortisone in high concentration thus increases the pre-incubation effect already observed by Clark and Walsh[1], although its action on the reticulocytes is slow. It also causes a change in certain permeability properties but no change in oxidative respiration. It is not known whether hydrocortisone operates by means of the same mechanism as pre-incubation, or whether some other mechanism is involved. It may produce its effect (*a*) by causing the reticulocytes to mature more rapidly, thus decreasing the iron-59 requirement; or (*b*) by altering some components of the reticulocytes so that less iron can be accepted. A reduction in oxidative respiration would have been expected if the effect were an increase in the rate of maturation of reticulocytes, because such maturation is characterized by loss of the oxidative pathway. On the other hand, there is evidence that hydrocortisone affects the permeability of reticulocytes.

Reticulocytes therefore differed from rat liver cells[2] in their reaction to hydrocortisone because (*a*) hydrocortisone must be used in high concentration before any effect is observed with reticulocytes, and (*b*) oxidative respiration of reticulocytes is not affected by hydrocortisone even in high concentrations. These differences are probably related to differences in metabolism and structure of the two types of cell.

This work was supported by a grant from the National Health and Medical Research Council of Australia.

Peggy Clark

New South Wales Red Cross
Blood Transfusion Service,
1 York Street, Sydney.

[1] Clark, Peggy, and Walsh, R. J., *Nature*, **184**, 1730 (1959).
[2] Gallacher, C. H., *Nature*, **182**, 1315 (1958).

Action of Methyl Hydroxybenzoate on Nervous Conduction

Methyl hydroxybenzoate and its sodium derivative are recommended by the B.P.C. and by Martindale[1] as preservatives for creams, emulsions, lotions and ophthalmic solutions, and they are used to preserve local anæsthetics. During an investigation of the action of phenol on nerve fibres[2,3], we examined the action of methyl hydroxybenzoate and its sodium derivative on conduction in fibres of the spinal roots of the cat, and also on the vagus and sympathetic nerves.

These substances, dissolved in Krebs's solution to form 0·1 per cent and 0·2 per cent solution, when applied directly to the spinal roots or to the vagus and sympathetic bundle in the neck, block conduction in nerve fibres of all sizes, myelinated and unmyelinated. The effect was typical of a local anæsthetic in that small fibres were blocked before large and that the conduction block was reversible. The blocking effect of a 0·1 per cent solution of methyl hydroxybenzoate was found to be similar to that of a 0·05 per cent solution of procaine.

The substances are recommended as preservatives, as, according to Martindale, they are "inert and non-toxic". The results mentioned above were obtained with the weakest solutions recommended by the B.P.C., the concentrations advocated being 0·1 and 0·2 per cent methyl hydroxybenzoate and 0·1–0·3 per cent of the sodium derivative.

The fact that in acute experiments conduction returned in fibres which had been blocked, following removal of the substance and washing the root or nerve bundle, cannot be taken to mean that no subsequent degeneration of the affected nerve fibres takes place. Exactly this situation occurs with phenol in non-aqueous solution. Although electrophysiological techniques have shown recovery of conduction in fibres which have been blocked by phenol, there is histological evidence of subsequent massive degeneration among these fibres[4]. If methyl hydroxybenzoate causes similar effects, it is possible that the degeneration of a proportion of nerve fibres following the injection of local anæsthetics in various painful states may account for the prolonged or

permanent relief of the pain that sometimes occurs. Whether this is the explanation of the prolonged therapeutic effect of local anæsthetics or not, it remains important to note that such recommended preservatives are not inert and that their presence should be taken into account both in experimental and in therapeutic situations.

P. W. Nathan
T. A. Sears

Medical Research Council
Neurological Research Unit
and Institute of Neurology,
National Hospital,
Queen Square, London.

[1] Martindale, W., *The Extra Pharmacopoeia*, twenty-fourth ed. (The Pharmaceutical Press, London, 1958).
[2] Nathan, P. W., and Sears, T. A., *J. Physiol.*, **150**, 565 (1960).
[3] Nathan, P. W., Sears, T. A., and Smith, M. C., *J. Physiol.*, **140**, 31, P (1958).
[4] Nathan, P. W., Sears, T. A., and Smith, M. C. (to be published).

HÆMATOLOGY

Alkali-resistant Hæmoglobins in Prosimian Primates

Prosimian Primates are a group of animals which share characteristics of behaviour, anatomy, biochemistry, cytogenetics, and physiology with the monkeys on one hand and the more primitive Insectivora on the other[1]. We have been examining the hæmoglobins of a number of these animals. One of our most interesting discoveries is the relatively high level of alkali-resistant hæmoglobin found in these animals.

Blood samples were taken from the femoral veins of anæsthetized or tranquillized animals. Hæmolysates were prepared as described by either Jonxis and Huisman[2] or Singer *et al.*[3]. The alkali-denaturation tests were carried out by the method of Singer *et al.*[3]. All samples were read in a Coleman Junior spectrophotometer at 540 mμ. Fresh and stored samples gave essentially identical results.

The results given in Table 1 include those from tests on individual adult and juvenile animals from a variety of genera of Primates. These results are of some comparative interest, since we have found no significant amounts of alkali-resistant pigments in adult animals in the Anthropoidea. There is a difference in average amount of alkali-resistant pigment among the three major groups of Prosimians tested. The Lemuriformes appear to have the highest average levels, 41–75 per cent; the Lorisiformes have levels between 8·4 and 52 per cent; and the single Tupaiiformes sample showed 9 per cent alkali-resistant hæmoglobin. The age of the animals apparently is not related to the amount of denatured pigment, since several in each group, except *Tupaia glis*, were infant or juvenile animals. This is in marked contrast to the higher Primates which have been examined. Singer *et al.*[3] found, in new-born human beings, a high level of alkali-resistant hæmoglobin (80 per cent); the level afterwards fell to the negligible value of 1·7 per cent at the age of 36 months. Similar values have been found in *Macaca mulatta*[4].

Electrophoresis on starch-gels of hæmoglobin from the animals studied here, with Poulik's discontinuous buffer system[5], demonstrated at least two components in all the Lorisiformes and in most of the Lemuriformes. One of these components may correspond to the alkali-resistant fraction we found.

Table 1

Animal	No. tested	Alkali-resistant hæmoglobin (per cent) Average	Range
Insectivora			
Elephantulus rufescens	1	0·4	
Primates			
Prosimii			
Tupaiiformes			
Tupaia glis	1	9·1	
Lemuriformes			
Lemur mongoz	2	75·5	68·1–82·3
L. fulvus	9	67·1	50·9–86·4
L. catta	6	61·8	51·1–71·5
L. variegatus	1	44·0	
L. macaco	2	41·5	39·5–42·7
Hapalemur griseus	1	67·0	
Lorisiformes			
Nycticebus coucang	2	52·5	49·5–55·5
Perodicticus potto	17	31·9	21·1–52·6
Galago crassicaudatus crassicaudatus	18	36·5	25·3–59·1
*G. crassicaudatus agisymbanus**	5	36·0	32·0–46·8
G. senegalensis senegulensis	4	8·4	7·2–10·1
Anthropoidea			
Erythrocebus patas	1	2·1	
Papio comatus	9	0·3	0·1–0·5
P. doguera	40	0·2	0–6·5
Macaca mulatta (fœtus)	2	82·0	75·9–87·6
M. mulatta (infant)	1	50·9	
M. mulatta (adult)	5	0·3	0–1·4
Cebus apella (infant)	1	10·8	
C. apella (adult)	1	2·6	
Lagothrix lagotricha	2	2·6	2·4–2·7
Ateles spp.	2	0·6	0·5–0·7
Pan troglodytes	1	0·2	

* Species identification in doubt. The animals appear to be of the *G. crassicaudatus* group; however, some authorities have called them *G. senegalensis zanzibaricus*.

A detailed account of the hæmoglobins of these Prosimians is in preparation.

One elephant shrew (*Elephantulus rufescens*) of the order Insectivora was examined. According to Polyak[6] *Tupaia* is closely related to this species. A level of about 0·4 per cent alkali-resistant hæmoglobin, which is negligible, was found in our sample from an old animal.

The question of the nature of this alkali-resistant hæmoglobin in the Prosimians is raised. There is little doubt that in higher Primates, including man, this hæmoglobin is the principal fœtal pigment. The term fœtal would be misleading, if not incorrect, when applied to the alkali-resistant fraction of Prosimian hæmoglobin. A possibility is that this hæmoglobin in the Anthropoidea was originally a major component of the respiratory pigment, as in the Prosimians, but gradually became confined to a shorter period in the animal's life, until, in the highest Primates, it is present only in the fœtus and during the first few months of life. This hypothesis requires an investigation into the physiological function of the alkali-resistant pigment in Prosimians.

Our work was supported by Research Grant 6053 of the United States Public Health Service, Research Grant 12331 of the National Science Foundation, and the research fund of the Department of Anthropology, Yale University. It is with pleasure that we acknowledge the assistance and encouragement of colleagues in various countries who helped us obtain the live animals used in this work.

John Buettner-Janusch
Joseph B. Twichell

Laboratory of Physical Anthropology,
Department of Anthropology,
Yale University,
New Haven, Connecticut.

[1] Andrew, R. J., *Behavior* (in the press).
[2] Jonxis, J. H. P., and Huisman, T. H. J., *Laboratory Manual on Abnormal Hæmoglobins* (C. C. Thomas, Springfield, Illinois, 1958).
[3] Singer, K., Chernoff, A. I., and Singer, L., *Blood*, **6**, 413 (1951).
[4] Sen, N. N., Das, K. C., and Aikat, B. K., *Nature*, **186**, 977 (1960).
[5] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[6] Polyak, M., *The Vertebrate Eye* (University of Chicago Press, Chicago, Illinois, 1955).

PATHOLOGY

Interaction of the Carcinogenic *N*-Methyl-Nitrosourethane with Sulphydryl Groups

In a preliminary communication I recently reported the production of lung adenoma in mice and squamous lung carcinoma in a rat, which was exposed to gaseous diazomethane. Among other rats given *N*-methyl-*N*-nitrosourethane (NMU) by stomach tube, a carcinoma of the stomach developed after a single dose of this compound[1].

Since this communication was published, more stomach tumours have been observed in the rats given NMU by stomach tube[2]. Stomach tumours have also been reported in rats given NMU in drinking water continuously until death[3]. Moreover, when NMU was administered to adult rats by a short mouse-stomach tube, the animals developed œsophageal squamous carcinoma[2]. Subcutaneous injections into mice gave rise to local sarcomata. It is evident that NMU acts at the site of application and that it is able to produce tumours in various tissues. This would qualify it to be considered a 'local' carcinogen.

However, the evidence described here indicates that the effective entity is not NMU but diazomethane, which is formed when NMU interacts with tissues. In 1895 von Pechmann[4] suggested that the toxic effects of NMU, which he observed on himself, might be due to diazomethane, formed from NMU in contact with tissues.

That this is really the case was qualitatively confirmed in the following way: NMU was added to tissue homogenates at 37° C. and the mixture was covered with a layer of ethyl ether. Rapid evolution of gas occurred; the gas bubbled through the layer of ether. This indicated that the gas is not soluble in ether, hence it is not diazomethane. Evidently, the diazomethane formed according to reaction (1) reacts immediately with tissue constituents, with evolution of nitrogen according to reaction (2):

$$\begin{array}{l} NO-N-CH_2 \\ \quad\quad | \\ \quad CO \longrightarrow C_2H_5OH + CO_2 + CH_2N_2 \\ \quad\quad | \\ \quad OC_2H_5 \end{array} \qquad (1)$$

$$CH_2N_2 \longrightarrow CH_2 \cdot\cdot + N_2 \qquad (2)$$

Expecting this to be an enzymatic reaction, it appeared convenient to study it in the Warburg apparatus, the likely final product being nitrogen. The other gaseous product of the reaction, carbon dioxide, is conveniently absorbed by 10 per cent potassium hydroxide, placed in the usual way in the middle well of the vessel. Tissue homogenates were prepared in Sørensen's phosphate buffer, *p*H 7·2 (1 : 10), and introduced into the main compartment of the Warburg flask (3 ml.). Solutions of NMU (1 : 100) were placed in the side-arm (0·5 ml.). (The solutions of NMU were freshly prepared from commercial *N*-methylnitroso-urethane by dissolving it in 50 per cent ethyl alcohol, 1 : 20; this gave a clear solution which was then diluted further with distilled water 1 : 5.) The effect of the buffer on NMU and of 10 per cent alcohol (0·5 ml.) on tissue homogenates was tested concurrently each time. The contents of the side-arms were added to the main compartment after 15 min. equilibration at 38·5° C. With tissue homogenates rapid gas evolution immediately followed the addition of NMU and was almost complete in 15 min.; the decomposition of NMU in buffer solution was very slow and slight gas evolution persisted for many hours.

When heparinized rat blood and plasma were tested in this way, rapid evolution of gas from NMU occurred only in the presence of blood corpuscles. The specific agent responsible for the decomposition of NMU is evidently cellular. The reaction was not affected by cyanide, azide or fluoride; it was, however, abolished by copper, indicating that SH-groups may be involved.

Indeed, when 1 per cent solutions of cysteine or glutathione in phosphate buffer at *p*H 7·2 were substituted for blood or tissue homogenates, the evolution of gas from NMU took place in the same way immediately after mixing. This proved that the reaction is chemical rather than enzymatic. Biotin, having sulphur in the ring, cystine, thioacetamide, or some representative cell constituents, such as glycine, tyrosine, guanine, pantothenic acid, etc., had no effect on NMU under the conditions tested.

The question arises: Are some of the SH-cellular compounds directly involved in the carcinogenic action of NMU, diazomethane and possibly of certain other alkylating agents? Diazomethane is known to be a very versatile reagent; it can methylate not only SH-groups but also any acidic or phenolic hydroxyls, amino- and imino-groups, it can add to double bonds, enlarge ring and carbon chains, form methyl branchings. It can conceivably form intra- and intermolecular methylene-bridges.

Which of the multiplicity of possible reactions is related to the carcinogenic action of this compound? The fact that decomposition of the strongly carcinogenic NMU takes place at the SH-groups supports the already existing evidence[5] that these may be involved in the carcinogenic process. However, if carcinogens combine with certain SH-compounds *in vivo*, the type of their binding is likely to differ fundamentally from that of non-carcinogenic agents, many of which are excreted as mercapturates. The action of carcinogens is irreversible and compatible with the survival of the affected cells; the interaction products possibly remain irreversibly *in situ*, and prevent the physiological replacement of some specific SH-compounds, the action of which they block.

Since this communication was submitted for publication, Emmelot and Mizrahi[6] reported that cysteine can counteract the action of dimethylnitrosamine. As the latter can act as a methylating agent (probably through the release of diazomethane after enzymatic oxidation)[7], these results confirm the view based on the more direct evidence here reported, that certain sulphydryl compounds are involved in the action of diazomethane and probably also of other carcinogens.

I wish to thank Miss A. Mackinnon for valuable technical assistance.

R. Schoental

Toxicology Research Unit,
Medical Research Council Laboratories,
Woodmansterne Road,
Carshalton, Surrey.

[1] Schoental, R., *Nature*, **188**, 420 (1960).
[2] Schoental, R., and Magee, P. N. (to be published).
[3] Druckrey, H., Preussman, R., Schmähl, D., and Müller, M., *Naturwiss.*, **48**, 165 (1961).
[4] von Pechmann, H., *Ber.*, **28**, 856 (1895).
[5] Crabtree, H. G., *Brit. Med. Bull.*, **4**, 345 (1947).
[6] Emmelot, P., and Mizrahi, I. J., *Nature*, **192**, 42 (1961).
[7] Hultin, T., Arrhenius, E., Löw, H., and Magee, P. N., *Biochem. J.*, **76**, 109 (1960).

Antagonism between Antibacterial Drugs

When two antibacterial agents are combined, the effect of the combination may equal the sum of the antibacterial effects of its components (addition), it may exceed this sum (synergism), or it may be smaller (antagonism).

According to Jawetz and Gunnison[1,2], the antibiotic and chemotherapeutic drugs can be divided into substances the effect of which is mainly bactericidal (penicillin, streptomycin, neomycin, and bacitracin) and substances that generally have only a bacteriostatic action (chloramphenicol, tetracycline, erythromycin and sulphonamides). Combinations of bactericidal substances often show synergism, but are never antagonistic. Antagonism is also extremely rare when two bacteriostatic agents are combined. But there is a chance of this type of interference when a bactericidal substance is combined with a bacteriostatic one.

Recently a more up-to-date schema, which also provides data about the latest accessions in the field of antibacterial substances, was developed in our laboratory. As a criterion of antibacterial potency the bactericidal action has been used. This criterion was applied by means of replica tests[3–7]; the standard of measuring the bactericidal action of the separate drugs and their combinations being not the diameter of the replica inhibition zones, but the number of colonies developing in these zones. The results have been arranged in the form of a schema.

The schema is based on numerous results obtained with some dozens of strains of *Staphylococci*, *Enterococci*, *Salmonellae* and *coli*-bacteria. More than 90 per cent of all strains investigated showed reactions as laid down in the schema. With streptomycin, bacitracin, novobiocin and the neomycin-group of antibiotics we noticed a slightly higher frequency of deviations. Our experiments confirm the conclusion arrived at by Jawetz and Gunnison that a fairly sharp distinction can be made between drugs acting *in vitro* mainly bactericidally (in the diagram enclosed by circles) and bacteriostatic drugs (in the quadrangles). Experiments with combinations of the drugs mentioned in the schema revealed that it is possible to distinguish four groups of agents: Group 1 (bactericidal substances): nitrofurans (nitrofurantoin, furazolidone, furaltadone), polymyxins (including colistin), streptomycin, neomycins (including kanamycin and framycetin), and bacitracin; Group 2 (bactericidal substances): penicillin, novobiocin, vancomycin, ristocetin and amphomycin; Group 3 (bacteriostatic substances): tetracyclines, chloramphenicol and the erythromycin group of antibiotics (erythromycin, magnamycin, spiramycin, oleandomycin); Group 4 (purely bacteriostatic substances): the sulphonamides and cycloserine.

With regard to the combinations of these substances the following points are worthy of note:

As a rule a substance belonging to one and the same group (that is, drugs in the same circle or quadrangle in the diagram) can be combined with another one of the same group without any risk of antagonism.

Drug antagonism is also only rarely encountered combining one of the bactericidal substances of group 1 with one of the substances of groups 2, 3 and 4. In these combinations the bactericidal agent generally strongly predominates and several of them even present clear cases of synergism (for example, streptomycin + penicillin, kanamycin + penicillin,

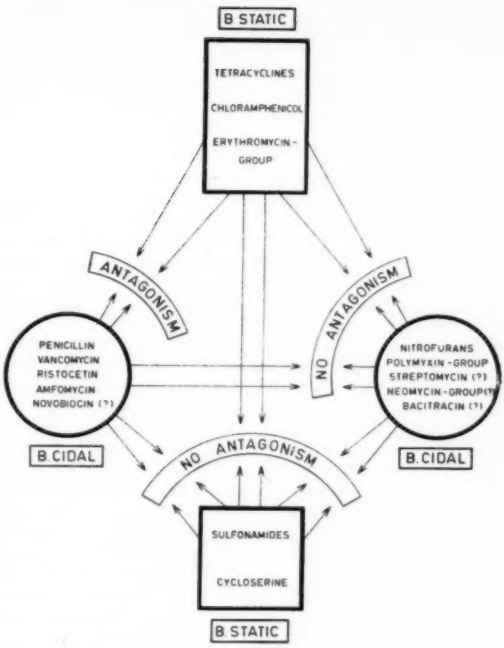


Fig. 1. Schema showing presence and absence of drug antagonism in pairs of antibacterial substances

and polymycin + chloramphenicol)[8–11]. Generally speaking, therefore, any drug from group 1 combines well with any one drug from the three other groups.

A quite different reaction occurs when a bactericidal antibiotic of group 2 is combined with a bacteriostatic drug of group 3. In these combinations the weaker, that is the bacteriostatic, drug dominates, so here there is antagonism. No such antagonism occurs when the drugs of group 2 are combined with those of group 4. Here the bactericidal drug predominates and sometimes there is even synergism (for example, penicillin + a sulphonamide[12]).

The different behaviour of the two groups of bactericidal substances (groups 1 and 2) is probably due to the different action of the drugs of these groups on growing and resting bacteria. It is a well-known fact that penicillin can only destroy bacteria when they are in the growing stage. The drugs of group 3 cause bacteriostasis and thus render penicillin ineffective. That the sulphonamides and cycloserine, which act also bacteriostatically, do not neutralize penicillin may be accounted for by the fact that a comparatively long space of time elapses before these drugs cause bacteriostasis. This interval lasts a few hours[13], which is amply sufficient to enable the relatively quick-acting penicillin to inflict irretrievable injury on the bacteria[14].

It is fairly safe to say that the bactericidal action of streptomycin, polymyxin and the nitrofurans is not to any large extent tied down to the growing stage of the bacteria[12,15,16]. Consequently, the bactericidal effect of these drugs is little impaired by bacteriostasis caused by one of the substances of groups 3 and 4.

On the other hand, the impairment of antibacterial action that occurs when a drug belonging to group 2

is combined with one from group 3 is not so negligible. The extent to which it affects the bactericidal action of penicillin in combination of this antibiotic with chloramphenicol, tetracycline, and erythromycin, was made the subject of a closer investigation. This revealed that, with regard to *Staphylococci*, the antagonism between penicillin and the other antibiotics manifests itself over a wide range of concentrations, from at least 0·1 to 10 units of penicillin per ml. Varying the concentration of the bacteriostatic antibiotics from 1 to 100 times the minimum inhibitory level did not cause the antagonism to disappear. What we also found was that the antagonism only affects the bactericidal potency of penicillin, the relatively weak bactericidal potencies of chloramphenicol, tetracycline, and erythromycin remaining unimpaired. In these cases, therefore, the type of antagonism encountered is strictly unilateral and only manifests itself in the reduced antibacterial action of one of the two components of the mixture.

In the above instances the antagonism results in the antibacterial potency of the stronger component of the combination being destroyed or lowered. Sometimes, however, it is the weaker component that is robbed of its effect by the other agent. This type of antagonism occurs when, for example, procaine penicillin is combined with a sulphonamide. Procaine, which is antagonistic to sulphonamide, cancels the action of this bacteriostatic, and, therefore, weaker agent. But on the other hand, a sulphonamide cannot impair the effect of the bactericidal, and therefore stronger, substance penicillin. Hence this weaker form of antagonism is also unilateral.

A. Manten
M. J. Wisse

National Institute of Public Health,
Utrecht, The Netherlands.

[1] Jawetz, E., and Gunnison, J. B., *Antib. Chemother.*, **2**, 243 (1952).
[2] Coleman, V. R., Gunnison, J. B., and Jawetz, E., *Proc. Soc. Exp. Biol. Med.*, **83**, 668 (1953).
[3] Lederberg, J., and Lederberg, E. M., *J. Bact.*, **64**, 29 (1952).
[4] Elek, S. D., and Hilson, C. R. F., *Brit. Med. J.*, ii, 1298 (1953).
[5] Manten, A., *Antib. Chemother.*, **4**, 1228 (1954).
[6] Manten, A., *Antib. Chemother.*, **6**, 480 (1956).
[7] Chabbert, Y. A., and Patte, J. C., *App. Microbiol.*, **8**, 193 (1960).
[8] Voorde, H. v. d., *Belg. Tijdschr. v. Geneesk.*, **4**, 175 (1960)
[9] Jawetz, E., Gunnison, J. B., and Coleman, V. R., *Science*, **111**, 254 (1950).
[10] Manten, A., and de Nooy, J. A., *Ant. v. Leeuwenh.*, **22**, 231 (1956).
[11] Manten, A., and de Nooy, J. A., *Ant. v. Leeuwenh.*, **25**, 183 (1959).
[12] Ungar, J., *Nature*, **152**, 245 (1943).
[13] Winkler, K. C., in *Leerboek der Microbiol. en Immunol.*, third ed., 349 and 370 (Utrecht, 1957).
[14] Cooper, P. D., *Bacteriol. Rev.*, **20**, 28 (1956).
[15] Asnis, R. E., and Gots, J. S., *Arch. Biochem.*, **30**, 25 (1951).
[16] Newton, B. A., *J. Gen. Microbiol.*, **10**, 491 (1954).

New Renal Tubular Amino-Acid Transport System and a New Hereditary Disorder of Amino-Acid Metabolism

We have investigated the metabolism of amino-acid in a kindred with familial nephritis and deafness. Renal hypoplasia and photogenic epilepsy, both familial, and a third independent familial disorder affecting L-proline metabolism were also found in this kindred. Familial nephropathy with deafness was recently reviewed[1], but its occurrence with these disorders was not described.

Chromatographical methods were used for qualitative[2,3] and quantitative[4,5] analyses of plasma and urine amino-acids in the father, mother, and six offspring including the male propositus, one male and four female sibs. The following results were obtained:

Plasma. Fasting L-proline concentration was significantly elevated above the accepted normal range (1·5–3·4 mgm. per cent)[6,7] on one or more observations, in the propositus and three affected female sibs (Table 1); consistent changes in the concentrations of other amino-acids were not detected in these subjects. Plasma amino-acids were normal in other kindred patients investigated.

Urine. Three subjects with abnormal concentrations of plasma L-proline also exhibited an amino-aciduria involving glycine, hydroxyproline and proline; the latter two amino-acids are not present in normal urine after the first few months of life[8]. A patient, with less abnormal plasma proline concentration, excreted only glycine in excess (Table 1). The mechanism of the amino-aciduria has been investigated. Fasting amino-acid renal clearance-rates were determined in one affected female sib by the methods described by Cusworth and Dent[6], before and after intravenous infusion of L-proline (71 mgm./kgm.).

Glycine and hydroxyproline clearance-rates were greatly increased while their own plasma concentrations remained normal; their clearance-rates were higher still when plasma proline concentration was further increased (Table 2). Urinary proline excretion, on the other hand, required an elevated plasma proline concentration.

Table 1. Fasting Plasma and Urine Proline, Hydroxyproline and Glycine Concentrations in Siblings with Hyperprolinæmia

Subject	Sex	Age (yr.)	Plasma proline* (mgm. per cent)	Urine amino-acids (mgm. amino-acid/100 mgm. total nitrogen†)		
				Proline	Hydroxy-proline	Glycine
C. M.	F	13	6·6	0	0	2,010
S. M.	F	12	6·4	—	—	—
			11·3	—	—	—
F. M. (propositus)‡	M	5	20·1	1,875	389	4,910
			7·8	406	42	489
M. M.	F	3	9·9	307	100	2,350
			7·5	0	0	613
			15·5	235	28	1,700

* Range of normal values (plasma) (refs. 6 and 7): proline, 1·5–3·4 mgm. per cent.
† Range of normal values (urine) (ref. 8): proline 0, hydroxyproline 0, glycine 537–1,371. Adult values valid for children beyond infancy.
‡ Patient has renal failure with reduced filtered load of amino-acids.

These results imply that glycine and hydroxyproline re-absorption in the renal tubule was impaired and that increased proline re-absorption (secondary to excessive filtration) was a determining factor for the amino-aciduria: other amino-acid clearance-rates were not significantly affected in this work.

Six normal subjects were also investigated. Fasting amino-acid clearance-rates were determined before (30-min. periods) and after (consecutive 15-, 30- and 30-min. periods), separate acute (3 min.) intravenous infusions of equimolar amounts of L-proline (71 mgm./kgm., five subjects), hydroxy-L-proline (82 mgm./kgm., two subjects), and glycine (48 mgm./kgm., three subjects). Table 2 also presents results on one of the subjects who received all three infusions. L-proline or hydroxy-L-proline infusion increased the clearance rates of the other two members of the triad. Glycine infusion produced similar but less impressive results. Clearance-rates of other amino-acids were relatively unaffected. Complete results on normal and affected subjects will be presented elsewhere.

These results suggest that glycine, L-proline, and hydroxy-L-proline share a common transcellular

Table 2. Fasting Amino-acid Infusion Investigations

Subject	S. M. hyperprolinæmic		C. S. normal		C. S. normal		C. S. normal	
Infusion $\frac{uv}{p}$ = $\frac{\mu gm./min./1{\cdot}73\ m.^2}{\mu gm./ml.}$ *	L-Proline (71 mgm./kgm.)		L-Proline (71 mgm./kgm.)		Glycine (48 mgm./kgm.)		Hydroxy-L-proline (82 mgm./kgm.)	
	Before (control)	After (45–75 min.)	Before (control)	After (0–15 min.)	Before (control)	After (0–15 min.)	Before (control)	After (0–15 min.)
L-proline $\frac{uv}{p}$	$\frac{11{\cdot}4}{113{\cdot}1}$	$\frac{948{\cdot}9}{184{\cdot}9}$	$\frac{0}{14{\cdot}3}$	$\frac{(—)}{139}$	$\frac{0}{20{\cdot}7}$	$\frac{5{\cdot}7}{22{\cdot}5}$	$\frac{0}{21{\cdot}0}$	$\frac{56{\cdot}4}{26{\cdot}0}$
Glycine $\frac{uv}{p}$	$\frac{197}{15{\cdot}2}$	$\frac{467{\cdot}2}{16{\cdot}2}$	$\frac{53{\cdot}0}{18{\cdot}3}$	$\frac{323}{17{\cdot}1}$	$\frac{88{\cdot}0}{20{\cdot}2}$	$\frac{(—)}{121{\cdot}7}$	$\frac{80{\cdot}0}{13}$	$\frac{231{\cdot}2}{16{\cdot}0}$
Hydroxy-L-proline $\frac{uv}{p}$	$\frac{17{\cdot}4}{0}$	$\frac{61{\cdot}9}{0}$	$\frac{0}{0}$	$\frac{414}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{(—)}{165{\cdot}0}$

* Normal range (ref. 6); proline $\frac{0}{15–34}$; glycine $\frac{49–116}{10–22}$; hydroxyproline $\frac{0}{0}$; (—) = immeasurable peak (infused amino-acid)

transport system in the renal tubule; the system has higher preference for the two amino-acids than for glycine.

The cause and significance of the hyperprolinæmia in the affected patients is under investigation; investigations of the full pedigree indicate its inheritance by a recessive mode, independent of the other familial disorders.

This work was supported by grants from the United States Public Health Service (*RG*–6742), and the National Heart Institute (*H*4706), from the United Cerebral Palsy Research and Educational Foundation, and the John A. Hartford Foundation. One of us (C. R. S.) was a McLaughlin Travelling Fellow and performed some of these investigations in the laboratory of Dr. C. E. Dent, University College Hospital Medical School, London.

C. R. Scriver

Montreal Children's Hospital,
Montreal 25, Canada.

I. A. Schafer
M. L. Efron

Children's Medical Centre,
Boston.

[1] Marie, J., *et al.*, *Annales de Pediatrie*, **36**, 84 (1960).
[2] Dent, C. E., in *Recent Advances in Clinical Pathology*, second ed., 238 (Churchill, 1951).
[3] Efron, M. L., *Biochem. J.*, **72**, 691 (1959).
[4] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **211**, 893 (1954).
[5] Moore, S., Spackman, D., and Stein, W. H., *Anal. Chem.*, **30**, 1185 (1958).
[6] Cusworth, D. C., and Dent, C. E., *Biochem. J.*, **74**, 550 (1960).
[7] Efron, M. L., and Scriver, C. R. (unpublished results).
[8] Jagenburg, O. R., *Scand. J. Clin. Lab. Invest.*, **11**, Supp. 43 (1959).

BIOLOGY

Serological Relationships between the Nine-spined and the Three-spined Stickleback

In zoological literature we can find some publications[1,2] in which the nine-spined stickleback, *Pungitius pungitius* (L.), is considered as belonging to the genus *Gasterosteus* L. But most contemporary zoologists consider that this species belongs to *Pungitius* Coste. This difference in attitude has encouraged me to undertake investigation on serological relationships between the three-spined stickleback, *Gasterosteus aculeatus* L., and the nine-spined stickleback, *Pungitius pungitius* (L.).

In my investigations I have applied the method proposed by Taliev[3,4] based partly on his own observations and partly on the results of other work.

To get the antigen and the antiserum, I used sticklebacks collected from the Olechówka River (near Łódź on July 20, 1960). The whole length of the body of fifty-six specimens investigated was 42–57 mm. The sticklebacks, without food for 24 hr., were ground into a mass after they had their heads cut off and their intestines and ribs cut out. I used this method because the small body of the stickleback does not permit one to get sufficient quantity of blood[5] necessary to produce the antigen and the antiserum. I fixed the mass with Ringer–Lock's tincture in the proportion 1 : 3; then I filtered it through infusorial earth. I used the product of filtering as an antigen and a vaccine to immunize rabbits. After the immunization I took blood directly from the heart of the rabbits. Having cleared plasma[6] from the morphotic elements, I fixed it with Ringer–Lock's tincture in the proportion 1 : 1, thus obtaining the antiserum. In a similar way I obtained the antigen from the nine-spined stickleback. The nine-spined sticklebacks (25 in number) were caught in the Bobrówka River, district of Łowicz, on 23.9.1960. The length of their bodies was 40–61 mm. In applying serology to establish the relationships among species[7], I also prepared the antigens from grown three-spined sticklebacks collected from the Bobrówka River (23.9.1960), the length of body being 45–53 mm., and from the young three-spined sticklebacks of this year (from the Olechówka River, 19.7.1960), the length of body being 25–30 mm.

In my work I applied the ring precipitation technique. I diluted the antigens as well as the antiserum with Ringer–Lock's tincture. I used the antiserum in constant concentration, 1 : 5. To interpret the results obtained I applied Boyden's method in which the positive reaction of the lowest concentration of an antigen on an antiserum obtained for it is accepted as 100 per cent.

As seen from Table 1, the serological distance between *Gasterosteus aculeatus* L. and *Pungitius pungitius* (L.) is great and it amounts to 56·3 per cent. The results obtained by me are approximately the same as those obtained by Taliev when examining the degree of serological relationship of many genera in the Cottoidae and the Salmonidae[8].

Table 1

Antigens from :	Antiserum against *Gasterosteus aculeatus* L. from the Olechówka River in the concentration of 1 : 5	
	The lowest concentration of the antigen with positive reaction	Percentage scale Boyden (per cent)
1. *G. aculeatus* L. from Olechówka River	1 : 800	100
2. *G. aculeatus* L. from Olechówka River, juv.	1 : 300	37·5
3. *G. aculeatus* L. from Bobrówka River	1 : 800	100
4. *P. pungitius* (L.) from Bobrówka River	1 : 350	43·7

The fact that identical results are found for the same species taken from different fishing places supports the application of serology to taxonomic investigations. The non-typical reaction with antigen of young three-spined sticklebacks is also in agreement with work already published.

Tadeusz Penczak

Department of Taxonomic Zoology,
University of Łódź, Poland.

[1] Leiner, M., *Z. Morph. u. Ökol.*, **28**, 108 (1934).
[2] Schindler, O., *Unsere Süsswasserfische*, 160, edit. by Kosmos, F. V. (Stuttgart, 1953).
[3] Taliev, D. N., *Trav. Stat. limn. Bajkal* (Leningrad), **10**, 268 (1940).
[4] Taliev, D. N., *Trav. Inst. Zool. Acad. Sci.* (Moscow), **8**, 43 (1946).
[5] Pora, E. A., *Inst. Oceanograph.* (Monaco), **699**, 1 (1936).
[6] O'Rourke, F. J., *Nature*, **183**, 1192 (1959).
[7] O'Rourke, F. J., *Irish J. Med. Sci.*, 77 (1960).

Biotin, a Growth Requirement for Four Soil Amœbæ

Subsequent to the publication of Adam's defined medium for the growth of Neff's *Acanthamoeba* sp.[1], I attempted to grow *Hartmannella rhysodes* in this medium slightly modified in the concentrations of amino-acids[2]. A further modification of Adam's medium was also tried in which all the amino-acids used were natural isomers (L forms), except threonine, valine and serine which were used as racemic mixtures (DL forms), and the methionine concentration was halved (13·5 mgm./100 ml.). All organic nutrients were obtained from the California Corporation for Biochemical Research (Los Angeles); the amino-acids used were 'A grade' except DL-isoleucine ('C grade'). The culture methods have been described previously[3]. Both modifications of Adam's medium failed to support growth of this hartmannellid amœba past the second or third serial culture using either glucose or sodium acetate as the carbon source. When biotin was added (0·5 μgm./100 ml.), satisfactory growth was obtained in serial cultures. The average maximum yield for duplicate cultures in the defined medium, containing 0·1 *M* glucose and grown at 30° C. on a rotary shaker, was $5{\cdot}76 \times 10^6$ organisms/ml. and the mean generation time was *c.* 40 hr. This is comparable to growth in the proteose peptone (Difco, Detroit)–glucose medium[2]. Identical results have been obtained with Neff's *Acanthamoeba* sp., *Acanthamoeba castellanii* and *Mayorella palestinensis* in both modifications of Adam's medium with either glucose or sodium acetate as the carbon source. Therefore all four soil amœbæ appear to require biotin in addition to the vitamins already present in Adam's medium (thiamine and B_{12}).

In order to establish whether biotin is a true requirement for the growth of these amœbæ or an artefact peculiar to the defined medium in my laboratory, I used 'Avidin' to bind biotin[4] in the proteose peptone–glucose medium (PPG) described previously[3]. 'Avidin' (Nutritional Biochemicals Corporation, Cleveland, containing *c.* 1,000–2,000 units/gm.) was added to the PPG medium in a concentration of 4 mgm./100 ml. The 'Avidin'-treated medium (PPG–A) and the controls were filter-sterilized. All four soil amœbæ mentioned above were carried in serial cultures of the PPG–A medium. Both the PPG medium and the PPG–A medium, supplemented with enough biotin to saturate the added 'Avidin' (10 μgm./100 ml.), were used as controls. At each subculture of the serial cultures explants were made into both control media. The PPG–A medium failed to support the growth of all four soil amœbæ past the second or third serial culture, whereas growth occurred in the explant controls. The experiments were carried out in duplicate on a shaker[3] and in stationary, slanted test-tube cultures at 30° C.

At present it is not possible to explain the apparent lack of a biotin requirement in the defined medium described by Adam[1,2] for Neff's *Acanthamoeba* sp. The observations given in the present report indicate that, in my laboratory, Neff's *Acanthamoeba* sp., as well as *Hartmannella rhysodes*, *Acanthamoeba castellanii* and *Mayorella palestinensis*, require biotin for growth in the defined medium and in the undefined proteose peptone–glucose medium.

This work was supported by grants from the Research Corporation (Brown-Hazen Fund) and the National Research Council of Canada.

R. N. Band

Department of Zoology,
University of British Columbia,
Vancouver, B.C.

[1] Adam, K. M. G., *J. Gen. Microbiol.*, **21**, 519 (1959).
[2] Adam, K. M. G. (personal communication).
[3] Band, R. N., *J. Gen. Microbiol.*, **21**, 80 (1959).
[4] György, P., in *The Vitamins*, **1**, 527 (Acad. Press, New York, 1954).

Effect of Ribonuclease on Shell Regeneration in *Ariophanta* sp.

It is supposed that the process of shell growth in molluscs consists of two phases. During the first step there is the elaboration of a protein membrane, and in the second mineralization of this membrane takes place[1,2]. Wagge has suggested that in *Helix aspersa*, which can rapidly repair its shell if this be damaged, the regeneration of the shell is brought about by the amœbocytes, which first lay down an organic framework that later on becomes calcified. Since ribonucleic acid is concerned with synthesis of proteins[3,4], which in this case form an integral part of the shell, we have tried to assess its role in the regeneration of the shell in the snail *Ariophanta* sp. (Pulmonata).

For experimental work adult snails were chosen, and into each was injected 0·5 ml. of distilled water containing 2 mgm. of ribonuclease (Armour, crystalline). Prior to injection, the enzyme solution was placed in a water-bath at 70° C. for 20 min. so as to destroy the proteolytic activity present in the ribonuclease preparation. The control specimens received 0·5 ml. of distilled water only. Pieces of shell (slightly less than 1 cm.^{2}) about 2 cm. away from the mouth of the shell were removed. Eighty snails were used, of which 40 served as controls. After injection, the animals were placed in glass bowls with wet filter papers at the top and bottom of the bowl so as to saturate the air with water vapour. They were not fed during the period of regeneration. Observations were made on the nature of the regenerating pieces at intervals of 24, 48 and 72 hr., after which parts of the shells with the regenerating pieces in the centre were cut from the specimens treated with ribonuclease and from controls. They were wiped dry and dehydrated in an air oven. The area of the regenerated piece was measured by an ocular reticule. Later, the regenerated piece was carefully cut out and weighed. From this, the weight of the regenerated piece per 1 cm.^{2} was calculated for both the treated and the control specimens.

Examination of the regenerated shell showed that the process of calcification was considerably inhibited

in specimens which had been treated with ribonuclease when compared with the controls even at the end of 72 hr. The dry weight of the regenerated piece at the end of 72 hr. in treated snails was 5·63 ± 0·16 mgm. per cm.², whereas in the controls it was 10·72 ± 0·40 mgm. per cm.².

According to Brachet[3] and Casperson[4], there exists a correlation between ribonucleic acid and protein synthesis; the former author[5] has shown that ribonuclease inhibits the incorporation of labelled phenylalanine into the proteins. Therefore, it is possible that ribonuclease, by acting on the ribonucleic acid in the amœbocytes, inhibits the synthesis of proteins which form the essential constituent of the organic framework of the shell.

The mineralization of this membrane is brought about by the amœbocytes which transport the calcium ions from the digestive gland and the shell. The inhibition in the calcification of the regenerating piece in the specimens treated with ribonuclease may be due to the inability of the amœbocytes to transport the necessary amount of calcium ions to the region of repair, since ribonuclease, by acting on the ribonucleic acid of the amœbocytes, prevents the binding of calcium ions at their cell surface. A similar inhibition in the uptake of calcium ions by *Elodea* cells in the presence of ribonuclease was observed by Lansing and Rosenthal[6].

It is suggested that ribonuclease, by inhibiting the synthesis of proteins which form an essential constituent of the shell and also by preventing the binding of calcium ions at the surface of the amœbocytes which normally transport calcium ions to the area of repair, slows down the process of regeneration.

Grateful acknowledgment is made to the Department of Atomic Energy, Government of India, for financial assistance.

K. K. Nair*
P. T. Muthe

Department of Zoology,
Wilson College,
Bombay 7.

* Present address: Biology Division, Atomic Energy Establishment Trombay, Bombay.

[1] Hirata, A. A., *Biol. Bull.*, **104**, 394 (1953).
[2] Wagge, L. E., and Mittler, T., *Nature*, **171**, 528 (1953).
[3] Brachet, J., *Arch. Biol.*, **53**, 207 (1941); *Nucleic Acids*, Academic Press, New York, 476 (1955).
[4] Casperson, T., *Naturwiss.*, **29**, 33 (1941).
[5] Brachet, J., *Nature*, **175**, 581 (1955).
[6] Lansing, A. I., and Rosenthal, T. B., *J. Cell. Comp. Physiol.*, **40**, 337 (1952).

Action of a Detergent (Dodecylbenzenesulphonate) on the Gills of the Trout

In the course of our work on the detrimental effect of surface-active agents on fishes we examined changes induced in the gills of the trout by sodium dodecylbenzenesulphonate (DBS). We found a reduction of the epithelium accompanied by a loss of the mucous cells on the top of the gill lamina starting at a concentration of 5 mgm. DBS/l. The same amount causes the respiratory folds of the lamina to stick together. An increasing destruction of epithelial cells follows the rise of concentration, until at 20 mgm. DBS/l. after 1 hr. the epithelium is destroyed to such an extent that the position of the respiratory folds is recognized only by the more resistant pillar cells. Multiple hæmatomas mark the destruction.

Fig. 1. *a*, Untreated control; *b*, concentration of DBS, 5 mgm./l., length of action, 8 hr.; *c*, concentration of DBS, 20 mgm./l., length of action, 30 min.

The deleterious action of DBS results in a diminished uptake of oxygen by the gills followed by death from suffocation when the concentration of DBS is strong enough or in case of prolonged action even in smaller amounts. The deterioration of respiration might be accompanied by a similar impairment of the salt balance and of the metabolism of urea since the gills take part in both mechanisms. Details of this work will be published in *Archiv für Fischereiwissenschaft*, **11** (1961).

O. J. Schmid

Physiologisch-Chemisches Institut der Universität,
Hamburg.

H. Mann

Institut für Küsten-und Binnenfischerei der Bundesforschungsanstalt f. Fischerei,
Hamburg.

The Species of the Teleostean Genus *Lebetus*

The genus *Lebetus* (Gobiidae) was founded by Winther[1] to include two species, *Gobius orca* and *G. scorpioides*, described by Collett[2] in 1874 from examples dredged on the Norwegian coast, and is characterized by the absence of an anterior membrane to the pelvic fins, which are united in the midline along their entire length. *Lebetus* occurs in the European Atlantic boreal region, being known from Iceland, Norway, the Skagerrak and Kattegat, the British Isles, and Brittany, but also extends into the Bay of Biscay and perhaps as far south as Gibraltar[3]. These fishes are of small size (up to 38 mm. total length[3]) and frequent rough grounds; in consequence they are not often taken, and are reputedly rare. However, in the past three years, more than fifty individuals of the genus have been obtained in the course of dredging off the southern end of the Isle of Man. This collection, together with earlier specimens from the same region and from several Scandinavian museums and the British Museum (Natural History), has provided considerably more material than has been available to previous workers on this genus.

Various characters have been used to identify the two species of *Lebetus*. Holt and Byrne[4] attached some importance to the coloration of the second dorsal fin. On this basis it has been possible to separate the above individuals into two groups,

termed *orca*, where this fin has a thick black superior edge above bright white areas and wide oblique bands of yellow (grey when preserved), and *scorpioides*, with a much thinner dark edge and four narrow lines of black and reddish orange across a predominantly hyaline fin. The absence from my collection of female *orca* and mature male *scorpioides* suggested connexion of the two coloration groups with sex and sexual maturity. Table 1 shows that mature or developing male *scorpioides* do not occur, and that female *orca* are unknown at any stage of maturation. This distribution would seem to indicate that the *orca* coloration is the male livery of a species the females and immature males of which bear the markings of *scorpioides*.

Table 1. Relation of Sex and Maturity to Coloration Groups

Group	No. of males (mature and maturing)	No. of males (immature)	No. of females (all stages)
orca	35	1	0
scorpioides	0	10	32

After reaching this conclusion, it was discovered that such a view was put forward by Smitt[5] in 1900, but was not accompanied by any details and appears to have been overlooked by later authors. The hypothesis is further supported here by the fact that the known examples of *orca* are 18·0–30·0 mm. in standard length whereas male *scorpioides* do not exceed 23·0 mm. Täning[6] has reported juvenile *orca*, but based his identification on meristic characters the validity of which is doubtful and is dealt with later. Female *scorpioides* attain the same maximum length as that of *orca*. Incipient development of the *orca* pattern in the second dorsal fin can be noted among some of the immature male *scorpioides*. The first dorsal fin in the *orca* fishes is a uniform yellowish grey edged with white, but in *scorpioides* is usually banded, with a dark spot on the interradial membrane between the fifth and sixth rays. This second pattern is stated to be found in the breeding male of *scorpioides*[4]; in fact, it is well represented in adult females, and among male *Lebetus* occurs only in the immature. Other aspects of coloration in which the two species have been regarded as differing can be seen in both *orca* and *scorpioides* groups.

Tests have also been made on other characters used in the past for distinguishing the two species.

Table 2. Means and Ranges of Values for Characters used in the Identification of *L. orca* and *scorpioides*, determined for the two Coloration Groups. A Linear Relation exists between Body Measurements and Standard Length

Characters used in identification	*orca* No. of obs.	*orca* Mean	*orca* Range	*scorpioides* No. of obs.	*scorpioides* Mean	*scorpioides* Range
*Meristic:						
No. of first dorsal rays	25	All 6		32	All 6	
No. of second dorsal articulated rays	25	9·2	8–10	31	9·0	7–10
No. of anal articulated rays	25	7·2	6–8	31	7·1	5–8
No. of pectoral rays	52	19·0	18–20	60	18·7	15–21
No. of caudal branched articulated rays	24	9·0	9–10	33	9·0	8–10
No. of scales in lateral series	38	26·7	25–29	41	26·8	25–29
Body proportions (in standard length):						
Depth at origin of pelvic fin	21	5·6	5·1–6·7	28	5·7	5·1–6·3
Depth at origin of anal fin	23	6·5	5·9–7·3	26	6·6	5·7–7·5
Depth (least) of caudal peduncle before origin of caudal fin	23	10·0	9·2–11·0	29	10·2	8·5–12·3
Distance from snout to anus	22	1·9	1·7–2·0	18	1·9	1·8–1·9

*British material only.

Diagnostic features attributed to *orca* have been an extra ray in the first dorsal fin, rather more articulated rays in the second dorsal and anal fins, but fewer in the pectoral fin and fewer scales in lateral series[4,6]. In body proportions *orca* has been regarded as consistently slimmer[4], and a supposed disparity in the position of the anus with reference to the middle of the body was utilized in a key by Smitt[7]. From Table 2 it is apparent that the two groups cannot be regarded as specifically distinct on these criteria. The wider range and slightly lower means for fin ray counts in *scorpioides* are due to the occurrence in this group of all the smallest fishes, among which such variation is more pronounced.

Another distinguishing character of *L. orca* is the greater size of the first dorsal fin, which is believed to differ from that of *scorpioides* in being considerably higher than the body[8], and higher than the second dorsal fin[3]. In the present work, length of fin ray has been used as an index of height of fin. With adults in both the *orca* and *scorpioides* groups, rays II and III of the first dorsal fin are longer than any from the second dorsal fin, and greater than the depth of the body at the origin of the pelvic fins. In length of ray II, there is little difference between the groups whereas for ray III and the other rays of the first dorsal fin, as well as in the second dorsal fin, there is a more or less pronounced divergence, *orca* having the longer rays. This is very noticeable in the last three rays (IV, V, and VI) of the first dorsal fin and, together with corresponding growth of the interradial and posterior membranes, gives the fin a much greater spread in the *orca* fishes.

Comparison of other characters, such as the patterns of sensory papillæ over the head and body, does not reveal any differences between *orca* and *scorpioides*. Both 'species' have been recorded throughout the geographical range of the genus, and, in so far as can be judged, display no ecological separation.

The two groups are thus different in coloration and in development of the dorsal fins, especially the first. As mentioned above, *orca* consists entirely of males, almost all maturing or mature, and *scorpioides* of females and immature males. The type specimens of *Gobius orca* and *G. scorpioides* (Zoological Museum, University of Oslo) have been examined, and are a male, maturing if not mature, and two females respectively. Sexual dimorphism in colour and length of fin ray is well known among teleosts, and has recently been discussed by Egami[9] for Japanese gobies. It seems safe to conclude, therefore, that the two names apply to one and the same species, for which the name *orca* Collett 1874 ranks by page priority as the senior synonym to *scorpioides* Collett 1874.

A fuller account of this work, and a re-description of the genus and species, are intended for publication elsewhere.

P. J. Miller

Marine Biological Station,
Port Erin, Isle of Man.

[1] Winther, G., *Naturh. Tidsskr. Kjöbenhavn*, (3), **11**, 41 (1877).
[2] Collett, R., *Ann. Mag. Nat. Hist.*, (4), **13**, 446 (1874).
[3] Duncker, G., *Tierwelt N.-u. Ostsee*, **12**, g, 121 (1928).
[4] Holt, E. W., and Byrne, L. W., *Rep. Sea Inl. Fish. Ireland 1901*, Pt. 2, 37 (1903).
[5] Smitt, F. A., *Öfvers. Svensk. Vet. Akad. Forh. 1899*, 543 (1900).
[6] Täning, A. V., *Vidensk. Medd. fra Dansk. Naturh. Foren.*, **104**, 267 (1940).
[7] Smitt, F. A., *A History of Scandinavian Fishes*, second edit. (Stockholm, 1892-95).
[8] Collett, R., *Forh. Vidensk. Selsk. Christiania 1874*, 151 (1875).
[9] Egami, N., *J. Fac. Sci. Univ. Tokyo*, Sect. 4, **9**, 67 (1960).

Manner of expressing Rate of Ribonucleic Acid Biosynthesis

ENRICHMENT of a medium supporting slow, balanced growth of bacteria (*Aerobacter aerogenes*[1] or *Salmonella typhimurium*[2]) is followed by a period of accelerating growth during which the ribonucleic acid (RNA) : protein ratio of the bacterial protoplasm increases considerably. It has recently been stated in papers from this laboratory[1] and elsewhere[2] that the rate of RNA synthesis during this adjustment period is even higher than it will be later when the new, high growth-rate is established. (The phrase, 'rate of RNA synthesis', refers to the rate of accumulation of total RNA, of which approximately 80 per cent is ribosomal. In the conditions of growth employed in the experiments being discussed, measurements of total accumulation are essentially measurements of ribosome synthesis, since turnover is slight.) This assertion is based on the fact that when the logarithm of RNA per ml. of culture is plotted against time, the slope immediately after enrichment of the culture is usually greater than that which will be maintained afterwards.

We wish to point out that the value obtained from such a slope represents the amount of RNA made per hr. per unit of RNA ($dR/dt \times R^{-1}$) and is not necessarily a meaningful expression of the rate of the biosynthetic process. In fact, unless the synthesis of RNA is truly an autocatalytic process, this manner of expressing its rate can be quite misleading. If alternatively, for example, one expresses the rate of RNA formation as the amount made per hr. per unit of protein ($dR/dt \times P^{-1}$), the data cited above (as well as new experiments to be reported[3]) give no indication of an overshoot in the rate of RNA synthesis by a culture in accelerating growth. Rather, the rate of RNA synthesis (expressed in this manner) simply increases, upon enrichment of the medium, to the value characteristic of the new growth-rate.

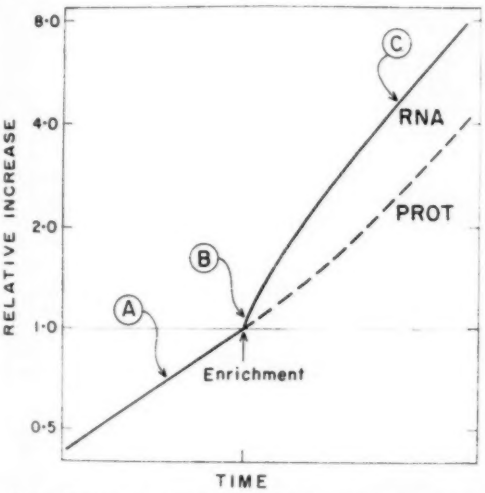


Fig. 1. Increase in ribonucleic acid (RNA) and protein (PROT) per ml. of culture before and after enrichment

The figure is idealized from data in the papers cited, and fulfils the following specifications: (1) The growth-rate finally reached after enrichment is exactly double that before. (2) The RNA : protein ratio at the higher growth-rate is double that at the lower. (3) The rate of RNA synthesis, expressed as the amount of RNA made per hr. per unit protein, increases instantly to the value characteristic of the higher growth-rate. Actually, such a curve may be determined simply by the third of these specifications, together with the assumption that the rate of protein synthesis (dP/dt) is proportional to the RNA content of the culture.

	$c_1 \cdot \frac{dR}{dt} \cdot \frac{1}{R}$	$c_2 \cdot \frac{dR}{dT} \cdot \frac{1}{P}$
A	1	1
B	4	4
C	2	4

To illustrate our point we have drawn an idealized curve which describes the accumulation of RNA and protein in such an experiment (Fig. 1). The rate of RNA synthesis, calculated per unit of RNA and per unit of protein, is given for three points : (*A*) balanced growth before enrichment ; (*B*) unbalanced growth immediately after enrichment ; (*C*) balanced growth after adjustment of the culture to the new medium. For purposes of comparison, each expression has been multiplied by a constant (c_1 or c_2) chosen so as to yield values at point *A* of 1. It is evident that the two ways of expressing the rate of RNA synthesis give qualitatively different impressions of what happens to this process following enrichment of the medium. Expressed per unit of protein, the rate of RNA synthesis quickly increases to the new higher value, while expressed per unit of RNA it overshoots (in this example by 100 per cent). The magnitude of the overshoot depends on the absolute increase in the growth-rate (over the range where RNA content of the bacteria is a function of the growth-rate) and on the rapidity with which the rate of RNA synthesis expressed per unit of protein reaches its new value.

Whether or not one concludes that RNA synthesis is faster during accelerating growth depends on how the kinetic data are expressed, and a choice between methods of expression must await elucidation of how RNA is made.

Our work is supported by the National Science Foundation under grant *G*-5887. One of us (D. G. F.) is a National Science Foundation Predoctoral Fellow.

D. G. FRAENKEL
F. C. NEIDHARDT
Department of Bacteriology and Immunology,
Harvard Medical School,
Boston 15, Massachusetts.

[1] Neidhardt, F. C., and Magasanik, B., *Biochim. Biophys. Acta*, **42**, 99 (1960).
[2] Kjeldgaard, N. O., Maaløe, O., and Schaechter, M., *J. Gen. Microbiol.*, **19**, 607 (1958).
[3] Fraenkel, D. G., and Neidhardt, F. C., *Biochim. Biophys. Acta* (in the press).

Effect of Temperature and Potassium Gibberellate on Phases of Growth of Algerian Ivy

THE application of gibberellic acid to mature, fruiting shoots of *Hedera* was found[1a,b] to cause a reversion from the shrubby fruiting form to the juvenile vining type of growth. Our experiments have confirmed this response but indicate that it depends on the maintenance of a sufficiently high temperature following treatment.

Plants of *Hedera canariensis* were treated in midsummer in a 60° F. minimum glasshouse with potassium gibberellate in concentrations of 0·0, 0·18, 0·91 and 1·8 mgm. a plant, applied in capillary melting point tubes inserted in decapitated stems, using ten plants per treatment. In 120 days, all the treated plants were juvenile, but only 30 per cent of the controls exhibited any juvenile characteristics. Identical treatments were applied in a lath-house outdoors in January in uncontrolled temperatures in which the minima ranged from 38° to 55° F. for most of the experiment. Although the plants grew

Table 1. Response of Algerian Ivy to Temperature and Potassium Gibberellate

Treatment	Temperature	Juvenile or transitional plants	Adult plants	Mean growth of longest shoot (cm.)
Potassium gibberellate	50°	0	24	27·5
	60°	19	4	34·8
	70°	21	1	50·1
	74°	18	2	35·1
Control	50°	1	22	12·2
	60°	3	20	16·7
	70°	6	18	19·1
	74°	6	18	15·5

well, none of the treated plants had reverted to a definitely juvenile condition and all the untreated plants remained fully adult after 120 days.

Since these observations indicated considerable difference in the ability of gibberellins to promote reversions at different seasons of the year, the effect of temperature was determined experimentally. Adult established plants, twenty-four in each treatment, were cut back to one shoot with one or two buds. A patch of bark was removed and 1 mgm. of potassium gibberellate was applied. All plants were placed under 16-hr. photoperiods at 1,000 ft.-c. using constant temperatures of 50°, 60°, 70° and 74° F. The reversions produced by the treatments together with shoot growth measurements are shown in Table 1. A few plants died and some of the forms classified as transitional had abnormal growth indicative of excessive concentrations of the chemical.

70° F. was the best temperature for elongation of shoots. Potassium gibberellate doubled the number of bud breaks at all temperatures and was effective in increasing elongation of shoots at all temperatures. Reversions were produced in all but the lowest temperatures. Several investigators have observed that higher temperatures favoured the production of juvenile growth, including Fisher[2] in *Ranunculus*, Hudson and Williams[3] in *Rubus*, and Njoku[4] in *Ipomoea caerulea*. Doorenbos[5] observed reversions on *Hedera* following grafting on juvenile understocks. Stoutemyer and Britt[6] verified this effect but showed that it depended on sufficiently high temperatures. The observation of Robbins[1b] that reversions were obtained with gibberellin more readily in summer than in winter may be explained as a response to temperature.

J. R. Goodin
V. T. Stoutemyer

Department of Floriculture and
Ornamental Horticulture,
College of Agriculture,
University of California,
Los Angeles.

[1] Robbins, W. R., (*a*) *Amer. J. Bot.*, **44**, 743 (1957); (*b*) **46**, 485 (1960).
[2] Fisher, F. J. G., *Nature*, **173**, 406 (1954).
[3] Hudson, J. P., and Williams, I. M., *Nature*, **175**, 814 (1955).
[4] Njoku, E., *New Phytol.*, **56**, 154 (1957).
[5] Doorenbos, J., *Proc. Kon. Ned. Akad. Wetensch.*, Ser. *C*, **57**, 99 (1954).
[6] Stoutemyer, V. T., and Britt, O. K., *Nature*, **189**, 854 (1961).

Effect of Gibberellic Acid, Kinetin and Other Substances on Seed Dormancy

The seeds of many plants, especially woody species from temperate regions, are dormant when harvested and require a period of chilling ('stratification') before they become capable of germination. As part of an investigation into the physiological basis of seed dormancy, attempts were made to replace the chilling requirement by treatment with gibberellic acid and other substances known to break dormancy. Gibberellic acid has been shown to stimulate germination in several members of the Rosaceae[1,2] and in various tree species[3–5].

The effects of gibberellic acid on dormant seeds of hazel, Kent cobs (*Corylus avellana*) and beech (*Fagus sylvatica*) have been examined. In germination experiments seeds were sown on filter paper or cotton wool soaked in distilled water or gibberellic acid solution and kept in the dark at room temperature. In unchilled nuts of beech and hazel only a few embryos were capable of germination, even after removal of pericarp and testa. When treated with 100 mgm./l. gibberellic acid, embryos and seeds germinated within 3 weeks. Beech embryos responded to a concentration of 1 mgm./l. In both beech and hazel, intact nuts failed to germinate, even in the presence of 500 mgm./l. gibberellic acid.

It was also confirmed that gibberellic acid will stimulate germination of birch seed in the dark[3], but that it has no effect on dormant sycamore seed[6]. Delayed epicotyl extension in acorns (*Quercus petraea*) was overcome by treatment with gibberellic acid.

It has been shown[2] that although gibberellic acid will accelerate germination of embryos from dormant Rosaceous seeds, it fails to have any effect when the coat structures are intact. This observation was confirmed with seeds of apple (*Malus* sp.), varieties 'Stoke Red' and 'Medaille d'Or', and rowan (*Sorbus aucuparia*). (See Table 1.) Gibberellic acid stimulated isolated embryos to germinate but had no effect if the endosperm was left intact, even if the testa had been removed. Concentrations as low as 1 mgm./l. failed to stimulate germination of embryos. In all the seeds used, coat structures prevented a response to gibberellic acid; in the case of beech and hazel it was the pericarp, whereas in the case of apple and rowan it was the endosperm plus testa which was effective. This lack of response could be due to (i) failure of the acid to penetrate the coat structures, or (ii) failure of embryos treated with gibberellic acid to overcome the retarding effect of the coat structures. If alternative (ii) was the case, then gibberellic acid only partially replaced the chilling requirement, since fully stratified seeds germinated despite the presence of coat structures.

Table 1. Effect of Gibberellic Acid on Apple and Rowan Seeds

	Untreated	Gibberellic acid (100 mgm./l.)
Apple var. 'Stoke Red' (1956)		
seeds	0	0
minus testa, endosperm intact	0	0
embryos	4	17*
,, chilled 10 weeks		
seeds	1	0
minus testa, endosperm intact	1	0
embryos	20	20
Apple var. 'Medaille d'Or' (1959)		
seeds	0	0
embryos	16	20
,, chilled 10 weeks		
seeds	18	—
Rowan (1960)		
seeds	0	0
embryos	1	17*

No. germinated out of 20. Apple after 1 week, rowan after 3 weeks.
* Significant effect at 99 per cent level of probability (using χ^2 test).

Hazel, beech and rowan seeds were treated with other germination-stimulating substances: potassium nitrate, thiourea and kinetin. Nitrate had no effect on hazel, beech or rowan. Thiourea and kinetin were both effective in stimulating germination in these species, although kinetin, at the concentration used (10 mgm./l.), had no effect on intact seeds (see Table 2).

Table 2. Effect of Various Substances on Dormant Seeds

	Untreated		Gibberellic acid (100 mgm./l.)		Kinetin (10 mgm./l.)		Thiourea (7·5 gm./l.)		Pot. nitrate (2 gm./l.)	
	2*	4	2	4	2	4	2	4	2	4
Beech nuts	0	0	0	0	0	0	0	0	0	0
seeds	0	0	5	16*	0	0	11*	13*	0	1
embryos	3	3	20*	20*	15*	16*	14*	15*	3	6
Hazel nuts	0	0	0	0	0	0	0	0	0	0
seeds	1	2	10*	20*	0	1	10*	19*	1	1
embryos	2	9	20*	20*	4	18*	8	18*	4	6
Rowan seeds	0	0	0	0	0	0	0	0	0	0
embryos	1	1	9*	19*	16*	19*	5	9*	0	1

2, No. germinated out of 20 after 2 weeks; 4, No. germinated out of 20 after 4 weeks.
* Significant effect at 99 per cent level of probability (using χ^2 test).

The effect of gibberellic acid on dormant seeds might be an indication that endogenous gibberellins play an important part in the chilling process. Kinetin and thiourea, however, both unrelated chemically to gibberellic acid, can also stimulate germination. Interest in the gibberellins lies in the fact that they have been shown to occur naturally in several seeds and that they promote other aspects of plant growth. Also gibberellic acid will overcome 'physiological dwarfing' in seedlings grown from unchilled embryos of Rosaceous seeds[2,7].

The work was carried out under the supervision of Prof. P. F. Wareing, part of it while I was supported financially by a grant from the Agricultural Research Council.

B. Frankland

Department of Botany,
University College of Wales,
Aberystwyth.

[1] Donoho, C. W., and Walker, D. R., *Science*, **126**, 1178 (1957).
[2] Nekrasova, T. V., *Fiziologiya Rastenii*, **7**, 106 (1960).
[3] Vaartaja, O., Canad. Dep. Agric., Forest Biol. Div., *Bimonthly Report*, No. 13, Part 2 (1957).
[4] Monin, J., Fourteenth Congrès des Sociétés savantes (1959).
[5] Villiers, T. A., and Wareing, P. F., *Nature*, **185**, 112 (1960).
[6] Phillips, I. D. J., Ph.D. thesis, University of Manchester (1959).
[7] Barton, L. V., *Contrib. Boyce Thomp. Inst.*, **18**, 311 (1956).

Bioassay of Gibberellic Acid using Excised Embryos of *Avena fatua* L.

A number of bioassays have been described for the detection of the gibberellins A_1 to A_9, particularly for gibberellic acid. Almost every assay so far reported for gibberellic acid is of the type which utilizes shoot growth in intact normal, or more often dwarf, species of plants. There is thus a need for alternative gibberellin bioassays utilizing other physiological responses. This communication demonstrates that the germination response induced in embryos of *A. fatua* L. treated with gibberellic acid provides an extremely sensitive and rapid micro-test for the acid.

The method used by us for assaying chromatograms, or small volumes of solution containing gibberellic acid, is as follows. Non-dormant *A. fatua* L. seeds are allowed to imbibe water for about 15 min. to soften the testa. Embryos are then excised with a sharp scalpel under a dissecting microscope leaving a thin layer of endosperm attached to the scutellum. When dried at room temperature these excised embryos can be stored for several months without affecting their response in the bioassay. Twenty-five dry embryos are placed on a standard sized paper (1 × 7/8 in. Whatman No. 1 chromatography paper) which is then wetted with 0·25 ml. of water (for chromatogram segments) or gibberellic acid (for assay of water solutions containing gibberellic acid).

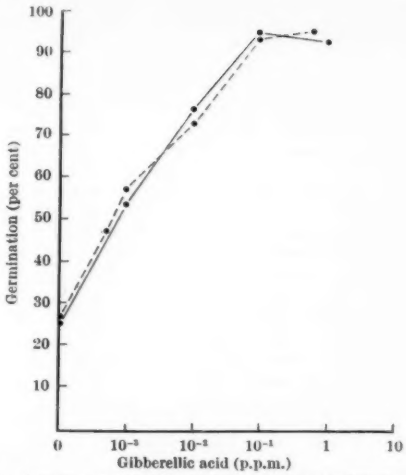


Fig. 1. Bioassay of gibberellic acid using the germination response of excised embryos of non-dormant *A. fatua* L. – – –, genetically heterogeneous population of seeds, germination recorded at 14 hr.; ——, seeds from an inbred line, germination recorded at 20 hr. Each point represents three replicates, each of 33 embryos. *S.E.* of all points is < ± 3

Each paper is kept in a small covered dish in a humid atmosphere at 20° C. in darkness. Each treatment is comprised of three such replicates. Germination percentages are scored by counting elongated radicles at intervals after 12 hr.

Two typical dose-response curves of non-dormant embryos to solutions of gibberellic acid are shown in Fig. 1. The optimal response to the acid lies between 10^{-1} and 1 p.p.m. It should be noted that concentrations lower than 10^{-3} p.p.m. (0·00025 μgm./replicate of 0·25 ml.) can be detected, and that up to 10^{-1} p.p.m. there is a linear germination response to the log concentration of dose of acid. There can be some advantage in using an inbred population of seed (solid curve) as opposed to a mixed population of seed (broken curve).

In our material, embryos which have not been after-ripened more than about 18 months show a degree of dormancy which, after maturation of the seed, decreases with period of after-ripening. During this period there is a distinct shift in the pattern of reaction; embryos respond to a lower concentration

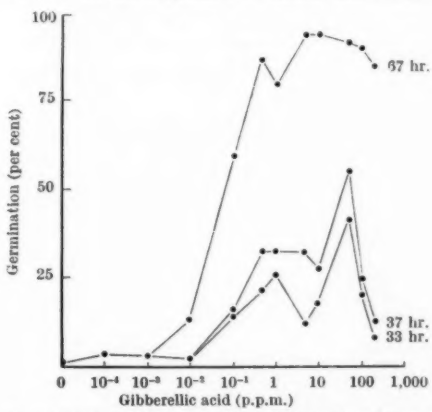


Fig. 2. Dose-response curves of semi-dormant embryos of *A. fatua* L. to gibberellic acid

of gibberellic acid. Also the time taken for germination to occur decreases considerably. Fig. 2 represents the response of seeds after-ripened for 4 months. It is apparent that the embryos show two responses to gibberellic acid with distinctly different concentration optima; the higher optimal concentration lying near 50 p.p.m. and the lower between 10^{-1} and 1 p.p.m. Moreover, if seed is mature but not after-ripened the embryos show a response only to the high range of concentrations (10^{-1} to 50 p.p.m.). An explanation of the physiological responses accounting for these differences has been presented in ref. 2. The response to high concentrations of gibberellic acid is known to be due to the ability of the acid to eliminate the requirement for exogenous sugar in germination.

Accordingly, the assay procedure is simplified by using embryos which have after-ripened to the point where exogenous sugar need not be supplied. However, the dose-response curve of dormant embryos to the acid in the presence of sugar is essentially the same as that of non-dormant embryos with the acid alone, with the exception that the time required for the assay is slightly longer for semi-dormant and substantially longer for very dormant seed.

Kinetin and indole-3-acetic acid (IAA) show no positive effects in the test. IAA induces some inhibition of germination at concentrations of 1–10 p.p.m., which, however, is reversed by gibberellic acid. The specificity of response of the embryos to different substances has not been otherwise investigated. It would appear that the cultivated wheat and oat varieties are unsuited for use as bioassay material as their response to low concentrations of the acid disappears fairly soon after the seed is matured.

In conclusion, the following points show some advantages of the *A. fatua* bioassay: (1) The assay can be completed in less than 18 hr. (2) Assay material occupies a small space with environmental conditions suited for routine laboratory testing, that is, a simple incubator. (3) Because material can be accumulated without loss of activity, large-scale assays are quite feasible. (4) Small volumes of solution (0·25 ml./replicate) together with low specific amounts (0·00025 μgm.) can be used for the assay. This can be important when dealing with extracts of plant material with low specific activity.

J. M. Naylor
G. M. Simpson

Field Husbandry Department,
University of Saskatchewan,
Saskatoon.

[1] Phinney, B. O., and West, C. A., *Ann. Rev. Plant Physiol.*, **11**, 411 (1960).
[2] Naylor, J. M., and Simpson, G. M., *Canad. J. Bot.*, **39**, 281 (1961).

ENTOMOLOGY

Occurrence of a Hyperglycæmic Factor in the Corpus Cardiacum of an Insect

Almost two decades have elapsed since Abramowitz *et al.*[1] first reported the occurrence of a 'diabetogenic' factor in the sinus gland of a crustacean. To the best of my knowledge the experiments described here are the first to show that a substance with a similar effect occurs in the corpus cardiacum of an insect. The corpus cardiacum was selected for investigation because of its analogy to the sinus glands of Crustacea and its known content of biologically active materials[2–4]. In view of the recent finding that the main blood sugar in insects is the non-reducing disaccharide trehalose[5–7] the effect of corpora cardiaca extracts on both reducing and non-reducing sugars in the blood has been investigated.

All observations were made on the adult male cockroach, *Periplaneta americana* L., 4–5 weeks after the final moult. Blood was removed from the cockroaches by the method in ref. 8 and tungstic acid filtrates prepared. Part of this filtrate was used for determination of reducing sugar, obtained by determining reducing value before and after fermentation with baker's yeast. Because trehalose yields glucose on hydrolysis it may be estimated as reducing sugar. Non-reducing fermentable values were obtained by hydrolysing a portion of the original filtrate with *N* hydrochloric acid at 15 lb./in.² for 30 min., neutralizing the hydrolysate with *N* sodium hydroxide and treating in the same manner as the unhydrolysed filtrate. The reducing power of the hydrolysed filtrate after correction for reducing power of unhydrolysed filtrate is considered to be a measure of blood trehalose. These values are expressed as mgm. per cent glucose equivalents. Reducing power was determined by the method of Somogyi as modified by Nelson[9].

The corpora cardiaca extracts were prepared by homogenizing a number of pairs of glands, from which the corpora allata had been removed, in a suitable volume of Pringle's insect Ringer. The extract was boiled for 5 min. and then centrifuged at 12,000*g* for 15 min. The supernatant was removed, diluted with Ringer solution to the desired concentration and stored in a deep-freezer until required for injection. Table 1 shows the effect of 10 μl. of extract, equivalent to one-tenth of a pair of glands, on the level of blood sugar at different times after injection. The control animals received Ringer solution only. It can be seen that except in two instances the level of reducing sugar was lower in those insects which received extract than in the controls. The effect of the extract on the trehalose content of the blood was very striking. 30 min. after injection it had risen 45·3 per cent and reached a maximum of 150 per cent 5 hr. after injection. Thereafter the trehalose content declined, returning to the normal level 48 hr. after injection. The figures for the control insects compare very well with those obtained for normal uninjected insects, the latter being 74 mgm. per cent reducing sugar and 1,174 mgm. per cent glucose equivalents of trehalose. When older (3 months) cockroaches were used the trehalose concentration was found to be of the order of 1,400 mgm. per cent glucose equivalents. This figure is in good agreement with that given in ref. 10. The extract was found to be effective even at a greater dilution than that used in the experiment described here, an injection equivalent to one-five-hundredth of a pair of glands increasing the blood trehalose by 30 per cent. When used in a concentration of one pair of glands/injection the mean increase in blood trehalose was 152·5 per cent, which is comparable to that obtained with one-tenth pair of glands, suggesting that one-tenth of the active material contained in

Table 1. Effect of Corpora Cardiaca Extracts on Blood Sugar
Figures are mgm. per cent glucose equivalents

Time after injection (hr.)	No. of insects	Reducing sugar		Trehalose		Increase (per cent)
		Control	Extract	Control	Extract	
0·5	12	25·2	31·4	1,237	1,797	45·3
1	12	70·3	31·8	1,225	2,583	110·8
3	12	29·7	41·7	1,293	2,558	97·8
5	12	37·2	29·3	1,050	2,625	150·0
12	12	66·1	29·1	1,118	2,178	94·8
24	12	43·4	32·3	929	1,941	109·0
48	12	52·4	19·5	1,025	1,160	13·2

the corpora cardiaca is sufficient to elicit the maximum response.

In order to determine whether the hyperglycæmic activity of the corpora cardiaca was specific to this tissue a number of other tissue extracts were prepared and tested for activity. The extracts, prepared from approximately 25 mgm. of tissue, were treated in the same manner as those of the cardiaca. The results of this experiment are shown in Table 2. All values, except one for brain tissue, indicate that none of the extracts had any effect on the level of blood trehalose. One of the values obtained for brain tissue was slightly high and may have been due to traces of cardiaca tissue remaining attached to the brain. The corpora allata were also examined for hyperglycæmic activity, but because of the technical impossibility of completely removing the corpora cardiaca, the results were inconclusive.

Table 2. Effect of Various Tissue Extracts on Blood Trehalose

Figures are mgm. per cent glucose equivalents

Time (hr.)	No. of insects	Control	Nerve cord	Muscle	Brain
5	6	1,241	1,041	1,147	1,470
5	6	1,119	1,218	1,069	1,263

The results of this work clearly show that the corpora cardiaca of the cockroach contain a powerful hyperglycæmic factor. Perhaps this is the 'insect adrenalin' described in ref. 3.

J. E. Steele

Pesticide Research Institute,
Canada Agriculture,
University Sub Post Office,
London, Ontario.

[1] Abramowitz, A. A., Hisaw, F. L., and Papandrea, D. N., *Biol. Bull.*, **86**, 1 (1944).
[2] Brown, F. A., and Meglitsch, A., *Biol. Bull.*, **79**, 409 (1940).
[3] Cameron, M. L., *Nature*, **172**, 349 (1953).
[4] Ozbas, S., and Hodgson, E. S., *Proc. U.S. Nat. Acad. Sci.*, **44**, 825 (1958).
[5] Howden, G. F., and Kilby, B. A., *Chem. and Indust.*, 1453 (1956).
[6] Wyatt, G. R., and Kalf, G. F., *Fed. Proc.*, **15**, 388 (1956).
[7] Evans, D. R., and Dethier, V. G., *J. Insect Physiol.*, **1**, 3 (1957).
[8] Sternburg, J., and Corrigan, J., *J. Econ. Entomol.*, **52**, 538 (1959).
[9] Nelson, M., *J. Biol. Chem.*, **153**, 375 (1944).
[10] Treherne, J. E., *J. Exp. Biol.*, **37**, 513 (1960).

Inhibited Oviposition by Females of *Gryllus assimilis* (F.), induced by Radioactive Males, using L-Methionine-Methyl-^{14}C

The taxonomy of North American field crickets of the genus *Gryllus* L. (until lately all referred to a single species, *Acheta assimilis* F.) has presented considerable problems in the past, and has recently undergone considerable revision[1–4].

In an attempt to help solve certain problems concerning speciation in this group of crickets, work with radioisotopes was initiated, using a laboratory culture of Jamaican *Gryllus assimilis* (F.), *sens. strict.*, and L-methionine-methyl-^{14}C. From these initial experiments an unexpected and interesting result was obtained. Not only did the treatment cause apparent sterility of the males, but also inhibition of egg-laying by females mated with radioactive males.

The experiments reported here were carried out in the Department of Entomology and Plant Pathology, Macdonald College, Ste. Anne de Bellevue, Quebec, in a greenhouse laboratory maintained at a temperature of 80° ± 1° F., and a relative humidity of 75 ± 5 per cent. The radioactive isotope, in distilled water, at dosages ranging from 0·05 to 10 μc./cricket, was injected into the hæmolymph of last-instar nymphs of both sexes of *G. assimilis* by means of an 'Agla' brand microsyringe (Burroughs Wellcome and Co., Ltd.). The needle was inserted laterally through the intersegmental membrane between the sixth and seventh abdominal segments.

At doses of up to 5 μc., the radioisotope had no deleterious effects on the insects and the nymphs metamorphosed to adult crickets and performed all functions of life quite normally, as did the controls which had been injected with equivalent quantities of distilled water alone. Higher concentrations of the radioisotope were lethal within 24 hr.

The nymphs injected with doses of 5 μc., or less, of the radioisotope all retained their radioactivity after moulting. The adult crickets thus obtained were then placed in individual 1-gallon, candy-jar breeding containers, provided, where appropriate, with oviposition cups after the manner described in ref. 5. All virgin females, both radioactive and normal (non-radioactive), began, on maturing, to lay numerous eggs whenever oviposition cups were available. This is usual in *G. assimilis* and certain other *Gryllus* and *Acheta* species, although parthenogenetic eggs have never been observed to develop. Also, when normal (non-radioactive) males were mated with either normal or radioactive virgin females, normal oviposition occurred. The eggs in both cases were viable, those from the radioactive females being also detectably radioactive as were first-instar nymphs which later hatched from them.

However, when radioactive males, derived from nymphs injected with any dose of L-methionine-methyl-^{14}C used, were mated with either radioactive or normal virgin females which had not yet begun laying, no oviposition occurred. Repeated experiments all produced the same results. Table 1 shows the average fecundity of radioactive and normal females, continually associated with normal and radioactive males. Figures for unmated virgin females (all of which oviposited) are also given by way of comparison. All radioactive individuals were derived from last-instar nymphs injected with L-methionine-methyl-^{14}C at a concentration of 1 μc./cricket.

Table 1. Results of Matings between Radioactive (*R*) and Normal or Non-radioactive (*N*) Individuals of *Gryllus assimilis* (F.) from December 22, 1960, to February 1, 1961

Matings		Average number of eggs per female
5 Males	5 Females	
R	*N*	0
R	*R*	0
N	*R*	196
N	*N*	126
—	*R*	123
—	*N*	90

It would seem from the results indicated in Table 1 that the radioisotope produced sterility in the males since no eggs were produced by the females with which radioactive males were associated. The urge to mate, however, was not impaired; the radioactive males mated as readily with both normal and radioactive females as did normal males; nor was the production of spermatophores inhibited. Judging from the average number of eggs laid per female, it would seem that the radioisotope may have led to an increase in the fecundity of females whether they had mated with normal males or not. This is indicated more clearly by the complete data than by the condensed table given here. It is also interesting to note that similar results have been obtained by administering the radioisotope orally via the drinking vials. Nymphs and adults were made radioactive by placing treated vials in the rearing jars for 72 hr.

It has, of course, long been recognized that male insects can be sterilized by irradiation with X-rays and/or γ-rays[6–9], but the present experiments appear to give the first indication that sterility may be induced by such a weak β-emitting radioisotope as L-methionine-methyl-^{14}C.

The second point of interest is that, whereas in previous cases of sterility in insects females have laid infertile eggs[7], in the case of *Gryllus assimilis* no eggs are laid by females after mating with radioactive males, even though unmated females normally lay (infertile) eggs. That radioactivity alone does not produce this result is indicated by the fact that radioactive females, either virgin or mated with normal males, oviposit normally; the implication is that some factor inhibiting oviposition is passed to the females from the radioactive males by way of the spermatophores (which were found to be detectably radioactive). It seems unlikely that non-radioactive L-methionine would have this effect, although this possibility is being explored.

Insufficient is known about the mechanism inducing oviposition in insects to indicate what might have occurred in this case, but it could be postulated that some reaction which permits or encourages the production of eggs when normal spermatophores are received by the female is blocked by the presence of a substance or substances originating from the radioactive spermatophores. That something of this kind is possible is indicated by the fact that inhibited females will begin to lay fertile eggs about 2 weeks after being mated with normal males. Similarly, such females isolated afterwards will begin to lay infertile eggs after about the same period. The fundamental question is posed: What causes oviposition to occur? Exactly what process is it that is inhibited by these hypothetical toxins from the radioactive spermatophores?

Whether there is any likelihood of a successful outcome from an attempt to put the type of sterility induced by radiation in *Gryllus assimilis* to practical use is doubtful. Bushland and Hopkins[7] used X-ray-sterilized males of the screw-worm fly, *Callitroga americana* (C. and P.), in the control of that insect in Florida, but this was made feasible because most females of this species mate only once. Females of *Gryllus assimilis*, on the other hand, mate frequently. The exact number of times has not been determined, but Spann[10] states that Kansas females of a related species (not *G. assimilis* although he uses this name) may mate about seven times daily. Thus, even though a radioactive male should inhibit oviposition in many females, it would not be long before the latter recovered and mated with normal males.

A. A. Abdel-Malek*
D. K. McE. Kevan

Department of Entomology and Plant Pathology,
Macdonald College,
McGill University.

* Permanent address: Department of Entomology, Faculty of Science, University of Cairo, Giza, Egypt.

[1] Alexander, R. D., *Ann. Ent. Soc. Amer.*, **50**, 584 (1957).
[2] Alexander, R. D., and Bigelow, R. S., *Evolution*, **14**, 334 (1960).
[3] Bigelow, R. S., *Canad. J. Zool.*, **36**, 139 (1958).
[4] Bigelow, R. S., *Canad. J. Zool.*, **38**, 509 (1960).
[5] Ghouri, A. S. K., and McFarlane, J. E., *Canad. Ent.*, **60**, 158 (1958).
[6] Runner, G. A., *J. Agric. Res.*, **6**, 383 (1916).
[7] Bushland, R. C., and Hopkins, D. E., *J. Econ. Ent.*, **44**, 725 (1951).
[8] Bushland, R. C., and Hopkins, D. E., *J. Econ. Ent.*, **46**, 648 (1953).
[9] Hassett, C. C., and Jenkins, D. W., *Nucleonics*, **10**, 42 (1952).
[10] Spann, L., *Trans. Kansas Acad. Sci.*, **37**, 299 (1934).

MICROBIOLOGY

Survival of *Rhizobium meliloti* in Soil Culture

The root nodule bacteria of leguminous plants (*Rhizobium* spp.) have repeatedly been observed to survive as long as 15–16 yr. in various media[1,2]. Bisset[3] has explained this remarkable longevity as due to formation of endospores in a complicated life-cycle.

The culture collection of this Laboratory includes many cultures of *R. meliloti* that have remained viable for much longer periods in a medium of loamy garden soil plus 0·5 per cent mannitol and some calcium carbonate, sterilized by autoclaving; the culture vessels are Freudenreich flasks that have been stored in darkness at room temperature, under which conditions the soil becomes air-dry within about a year. During the autumn of 1960 I examined such cultures of twenty *R. meliloti* strains, seventeen from lucerne and three from *Medicago lupulina*. The age of these cultures ranged from 30 to 45 years, the majority (13) being 38–40 years old.

A pure growth of *R. meliloti* developed from all cultures within a week when small quantities of soil were transferred to slopes of glucose–asparagine agar plus lucerne seedling extract. Plate counts on the same medium were rather erratic but suggested numbers from less than a hundred to about half a million per gm. of soil, with no apparent correlation between age and density of bacteria.

Some pasteurization tests were made on soil suspensions (one part of soil in ten parts of sterile tap water) heated for 5–30 min. in a thermostatically controlled water-bath; duplicate portions were cooled rapidly and ten drops of suspension were transferred to slopes of agar medium that were watched for growth at 25° C. for three weeks (in some cases for six weeks, which made no difference). Eleven strains failed to grow after heating for 5 min. at 50° C. The remaining nine showed somewhat inconsistent growth after heating for 5–20 min., one of them (*L.* 21, aged 39 yr.) even after 30 min. These strains, and five more, were tested at 60° C.; all succumbed after 5 min., except *L.* 21, which, in one instance, survived for 10 min. The rhizobia in the soil are thus about as heat-sensitive as in agar cultures[1], and according to this evidence they do not survive as thermoresistant endospores.

Nutman[4] found *R. trifolii* frequently producing ineffective variants when grown in sterile soil. To see if this had happened in the present cultures, 200 isolated colonies were picked from the plate counts (20 colonies from each of 10 strains) and the isolates were tested for effectiveness on lucerne seedlings in aseptic agar cultures. All isolates formed normal nodules with red pigment; nodule-bearing plants were dark green and healthy-looking and contained 2·7–4·6 per cent nitrogen in dry matter 8 weeks after inoculation, while the uninoculated control plants were dwarfed and yellowish, with 1·2–1·7 per cent nitrogen. One isolate (from strain *L.*4, aged 44 years) was ineffective immediately after isolation but was normally effective when re-tested a couple of months later. Formation of permanently ineffective variants in *R. meliloti* thus seems to be quite a rare occurrence.

The collection includes no cultures as old as those of *R. meliloti*, but soil cultures of several other organisms proved viable at ages of 10–14 yr. This applies to bacteria (*R. trifolii*, *R. leguminosarum*, *Pseudomonas*

aeruginosa) as well as actinomycetes (*Nocardia*, *Streptomyces*, *Micromonospora*) and fungi (*Trichoderma*, *Penicillium*, *Aspergillus*).

Sterilized soil as culture medium thus seems to keep vegetative cells of *R. meliloti* alive and effective in a desiccated condition for periods approaching half a century; moreover, it preserves for more than ten years the viability of several other micro-organisms that have never been claimed to form endospores.

H. L. Jensen

State Laboratory for Soil and Crop Research,
Lyngby,
Denmark.

[1] Fred, E. B., Baldwin, I. L., and McCoy, E., *Root Nodule Bacteria and Leguminous Plants* (Univ. of Wisconsin Studies in Science, No. 5, 1932).
[2] Allen, E. K., and Allen, O. N., *Ruhland's Handb. d. Pflanzenphysiol.*, **8**, 48 (1958).
[3] Bisset, K. A., *J. Gen. Microbiol.*, **7**, 233 (1952).
[4] Nutman, P. S., *J. Bacteriol.*, **51**, 411 (1946).

Inhibition of Lysozyme by 'Suramin'

It has been shown that a number of non-chaining bacteria form long chains when grown in the presence of 'Suramin'; the chains are rapidly split by filtrates of ordinary 'short form' cultures of the organisms. It was assumed as a working hypothesis that separation of bacterial cells is an enzymic process and that 'Suramin' acts by inhibiting the cell-separating enzyme[1].

Detailed experiments showed that while *Streptococcus faecalis*, *B. megaterium*, *Cl. welchii* and *Cl. sporogenes* grow well in the presence of 'Suramin' at a concentration of 1 gm. in 40 ml. (w/v), some chaining still occurs at concentrations up to 1 gm. in 5,000 ml. (w/v). The hypothesis of enzyme inhibition was confirmed when it was found that the chain-splitting activity of the filtrates was indeed inhibited by 'Suramin' at concentrations ranging from 1 to 15 μgm./ml. (w/v).

It is also known that chains of various aerobic and anaerobic rods (obtained by growth in magnesium-deficient medium) and chains of *Streptococcus pyogenes* can be split by lysozyme[2,3]. Preliminary experiments confirmed these findings and extended them to *Cl. sporogenes* and *Streptococcus faecalis*. Relatively high concentrations of lysozyme were required to split the chains in the standard time of 30 min. (20–100 μgm./ml. w/v); the splitting was inhibited by 'Suramin'.

Evidence was afterwards obtained (to be published in detail elsewhere) that despite the similarity of effect between lysozyme and filtrates, splitting of chains is not in fact caused by lysozyme but is due to an enzyme of bacterial origin—apparently released by lysozyme from the cells. Since, however, 'Suramin' is a powerful inhibitor of lysozyme it was thought interesting to give an account of it.

Crystalline lysozyme (Sigma Co.) and 'Suramin' (Imperial Chemical Industries) were sterilized by filtration through sintered glass. Lysozyme was assayed by estimating its ability to lyse suspensions of *Micrococcus lysodeikticus* (N.C.T.C. 2665). The organism was grown at 28° C. on meat-extract broth agar for 48 hr. and the cells washed three times with saline. Suspensions of 10^9 cells/ml. were made in saline-phosphate buffer *p*H 5·5, and falling amounts of lysozyme added. Results were read after 30 min. at 20° C. taking 50 per cent clearing of the suspension on an EEL nephelometer as the end point.

The results can be summarized as follows: lysozyme caused clearing of the suspensions of *Micrococcus lysodeikticus* up to a concentration of 0·1 μgm./ml. (w/v). 'Suramin' gave 50 per cent inhibition of lysozyme when present at equal weight. Accepting the mass of lysozyme as approximately ten times that of 'Suramin', inhibition occurred when 'Suramin' was present at a ten-fold molar excess.

In some experiments 'Suramin' was first left in contact with the bacterial cells; provided that 'Suramin' was afterwards removed by washing the cells with saline no inhibition was observed. In other experiments 'Suramin' was allowed to interact with lysozyme before the cell suspension was added; maximum inhibition was reached rapidly in 5–15 min.

We are indebted to the Rankin Research Fund for a grant towards the expense of this work.

Iwo Lominski
Samuel Gray

Department of Bacteriology,
The University,
Glasgow.

[1] Lominski, I., Cameron, J., and Wyllie, G., *Nature*, **181**, 1477 (1958).
[2] Webb, M., *J. Gen. Microbiol.*, **5**, 496 (1951).
[3] Welshimer, H. J., *J. Bacteriol.*, **66**, 112 (1953).

Dihydroxyacetone Production in *Acetomonas*

It has already been shown[1] that a useful primary criterion for differentiating *Acetomonas* strains from those of *Pseudomonas* is the ketogenic action of the former on glycerol which results in the production and accumulation of dihydroxyacetone. Thus the 165 strains of *Pseudomonas* studied all failed to show production of dihydroxyacetone, while 49 out of the 50 *Acetomonas* strains produced it abundantly.

As regards the single anomalous *Acetomonas* strain (*A. suboxydans* N.C.I.B. 3734) it was tentatively suggested that this might not be an *Acetomonas* strain at all, but a mutated *Acetobacter* strain which had possibly lost the typical ability of acetobacters further to oxidize the acetic acid produced to carbon dioxide and water, for production of dihydroxyacetone is much less common in *Acetobacter* than in *Acetomonas*. There was, however, no way of knowing whether it was originally dihydroxyacetone-negative when first isolated or whether it has lost dihydroxyacetone positivity in the course of long sub-cultivation.

However, we now have definite evidence that the ability to produce dihydroxyacetone can be lost by 'mutation' (in the broadest sense) in the course of laboratory sub-cultivation. A strain of *Acetomonas*, isolated from cider, was strongly dihydroxyacetone-positive when first isolated. Now, after sub-cultivation on apple juice agar for some two years, this strain is completely dihydroxyacetone-negative, although it has retained its original ability to produce deep brown to black water-soluble pigment on yeast extract/glucose/calcium carbonate agar.

It thus appears that production of dihydroxyacetone from glycerol, if negative, is not so conclusive as formerly in suggesting that a strain is a pseudomonad, but merely functions as a first presumptive indication to that effect. However, even if dihydroxyacetone-production is negative, the other three primary criteria[1] found always to be conclusive for differentiating *Acetomonas* from *Pseudomonas* strains

amply suffice to prevent any possibility of confusing these two completely different types of bacteria.

J. G. CARR
Department of Agriculture and Horticulture,
University of Bristol,
Research Station, Long Ashton,
Bristol.
J. L. SHIMWELL
Research Department,
British Vinegars, Ltd.,
Welshmill, Frome.

[1] Shimwell, J. L., Carr, J. G., and Rhodes, M. E., *J. Gen. Microbiol.*, **23**, 283 (1960).

CYTOLOGY

Chromosome Preparations obtained directly from Peripheral Blood

ABNORMALITIES of both number and morphology of the chromosomes of leukæmic cells in man have been reported[1,2]. All such work reported to date has been made on cells from marrow or from peripheral blood cultured *in vitro*. Such findings are open to the theoretical objection that any observed abnormality might have arisen during the culture process. In order to obviate this possibility, we decided to attempt to take advantage of the situation in leukæmia where the bone marrow is abnormally active, and later developed a method of making preparations without prior culture[3].

This method has now been successfully adapted for application to cells obtained directly from the peripheral blood in cases of leukæmia where again abnormal numbers of cells, many of them in division, may be found.

Venous blood (20 ml.) was collected into the dipotassium salt of (ethylene dinitrilo) tetraacetic acid. The white cells were separated from the red by centrifugation at 400 r.p.m. at 0–4°. The supernatant plasma and white cells were decanted and 0·72 per cent sodium citrate was added for 10 min. After centrifugation at 1,500 r.p.m. and resuspension of the leucocytes in 3 : 1 (ethanol : acetic acid), the material was hydrolysed in *N* hydrochloric acid at 60° C. for 4–6 min. (depending on the specimen) and stained with orcein.

Fig. 1. Direct peripheral blood preparation. Hyperdiploid metaphase ($2n + 1$) in a male with acute leukæmia. The additional chromosome is shown at *A*

It has been found that the dipotassium salt of (ethylene dinitrilo) tetraacetic acid used as an anticoagulant has a colchicine-like action on chromosomes, producing many well-differentiated metaphase plates. There is thus no need for previous administration of colchicine or colcemid. Very satisfactory preparations, suitable for both counting and morphological work, are being obtained by this simple method.

M. A. KINLOUGH
H. N. ROBSON
Department of Medicine,
University of Adelaide,
South Australia.

[1] Baikie, A. G., Court-Brown, W. M., Jacobs, P. A., and Milne, J. S., *Lancet*, ii, 425 (1959).
[2] Baikie, A. G., Court-Brown, W. M., Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Tough, I. M., *Nature*, **188**, 1165 (1960).
[3] Kinlough, M. A., Robson, H. N., and Hayman, D. L., *Nature*, **191** 420 (1961).

Surface Granulation in Myeloid Leucocytes

THE clinical use of cytotoxic drugs which have a more or less specific action on cells of the lymphoid or myeloid series has made more important the differentiation of one type of acute leukæmia from another. Any technique which points to distinct biochemical or physico-chemical differences between myeloid and lymphoid cells is therefore worthy of further study. This communication is concerned with distinct differences in these two types of cells revealed by certain effects of fixation. These have not been reported before.

In 1957 McNary[1] described the application to blood cells of a histochemical stain specific for zinc. The stain contains diphenylthiocarbazone (dithizone) dissolved in absolute acetone, water, 1 *N* acetic acid, sodium potassium tartrate, and a complex-forming buffer (sodium thiosulphate, sodium acetate, potassium cyanide and glacial acetic acid) at *p*H 5·5.

After staining blood smears for 15 min., neutrophil and eosinophil granulocytes, but not lymphocytes, show a golden-brown discrete 'granulation' in the cytoplasm which is easily seen with high-power magnification, provided a dry objective is used. Viewed under oil immersion, or after mounting, the 'granulation' is no longer visible. The same golden-brown 'granulation' is produced if dithizone is left out of the stain.

Accentuation of this effect is obtained with phase contrast and a dry objective × 45; the neutrophils contain numerous black dots and the lymphocytes contain none (Fig. 1*a*.) The black dots disappear when viewed through oil, and where the black 'granules' were previously seen the cytoplasm now shows fine patchy areas which are paler than the rest of the cell (Fig. 1*b*).

This phenomenon can be explained if the 'granules' are regarded as irregular elevations due to folding of the cell surface, resulting from the effects of *p*H and fixation. Light passing through a cell is altered in phase by an amount depending on the product of refractive index by thickness[2]. These elevations would therefore appear black under phase contrast because the cell is thicker in this region. Addition of oil with a greater refractive index than the cell would reverse the picture as there would be now less oil over the elevations than over the rest of the cell. In support of this concept, it is known that precipitant fixatives

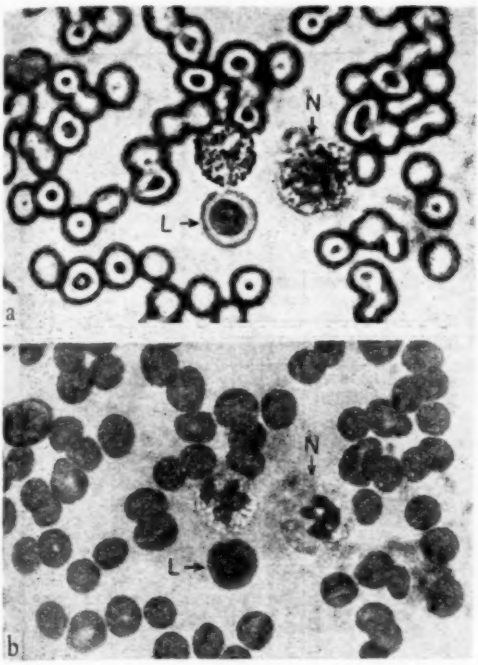


Fig. 1. *a*, Blood smear showing two neutrophils with 'surface granulation' (*N*) and one lymphocyte with clear cytoplasm (*L*). Counterstained with Leishman. Phase contrast × 450. *b*, Same field as in *a* but under oil immersion (× 450). Disappearance of 'surface granulation'

containing acetic acid often produce considerable distortion of the cell[3].

Sodium thiosulphate in the presence of acetone, water and acetic acid is effective in producing neutrophilic 'surface granulation', but the change is dependent on *p*H and is best developed at a *p*H near 5·5. This was determined by testing freshly prepared air-dried blood smears against the various components of McNary's stain. Neither dithizone, sodium potassium tartrate nor potassium cyanide was necessary for the production of 'surface granulation'. Absolute acetone, or acetone plus water, alone does not produce 'surface granulation' although it adequately fixes blood smears.

If blood smears are fixed first in methanol, formalin vapour, or osmium tetroxide vapour, neutrophils no longer show 'surface granulation'. Neutrophils from the dog do not show 'surface granulation' under conditions which produce it in human neutrophils.

The differences described here between human neutrophils and lymphocytes must reflect biochemical differences in cell proteins. These differences are being investigated in lymphocytic and granulocytic cells at various stages of maturation.

This work was supported by a grant from the Anti-Cancer Council of Victoria. I am grateful to Dr. T. E. Lowe for his help.

A. D. McCutcheon

Baker Medical Research Institute,
Alfred Hospital,
Melbourne, Australia.

[1] McNary, W. F., *Blood*, 12, 644 (1957).
[2] Barer, R., *Analytical Cytology*, Chapter 3, edit. by Mellors, R. C. (McGraw-Hill Book Co., New York, 1955).
[3] Strangeways, T. S. P., and Canti, R. G., *Quart. J. Micro. Sci.*, 71, 1 (1927–28).

ARCHÆOLOGY

Absolute Age of Pleistocene and Holocene Deposits in the Haua Fteah

The general characteristics of the large coastal cave site known as the Haua Fteah, in north-eastern Libya, have already been described, together with a brief résumé of the most important results obtained up to 1952[1,2]. A further season's work in 1955 provided additional fossil human remains associated with Middle Palæolithic occupation débris[3,4], enabled a much closer analysis of the stratigraphy, and, by opening up a wider area, made possible the excavation of a deep sounding into earlier cultural horizons than any hitherto examined, down to a total depth of 42·5 ft. In the past six years a detailed programme of study of the cultural, palæontological and other collections has been carried out in this Department, and further samples for carbon-14 analysis have been processed.

From an archæological point of view the site is now seen to offer a nearly continuous cultural succession from a pre-Mousterian stage (provisionally labelled Libyan Pre-Aurignacian by analogy with the "Praeaurignacien" identified by Rust[4] in Syria) up to the present. The stratigraphy containing this succession consists for the most part of remarkably regular horizontal layers, distinguished from one another by minor differences in colour and lithology, and ranging in thickness from about 6 to 18 in. (ref. 2). Mammal bones form a considerable proportion of the occupation debris and are the subject of a separate communication elsewhere[5]. The object of the present note is to make available, in advance, the principal data on which the absolute age of the cultural and biological succession is based, since these would appear to be relevant to other investigations being carried out elsewhere in the Mediterranean and adjacent areas.

Carbon-14 age estimates were obtained by H. E. Suess, at the Washington Laboratory, from samples collected in 1952 (ref. 6), and by the late Hl. de Vries, from samples collected in 1955 (personal communication). In the accompanying diagram (Fig. 1) these ages have been plotted against the cumulative depth obtained by adding together the maximum observed depth of each layer. This device has been adopted in order to minimize the effects of localized denudation, and hence approximate as nearly as possible to the relative time-interval represented by each layer.

It will be noted that as far back as 28,500 before the present the points appear to approximate to a logarithmic curve representing a gradually decreasing ratio of thickness to time unit. This is not unexpected since the effects of compression by increasing overburden, plus possible weathering *in situ* by percolating water, should in general lead towards progressive reduction in thickness.

Below a depth of about 25 ft., however, this tendency seems to be reversed by dates *W*85, *GRO* 2022, 2023, and a preliminary reading for layer XXIX, *GRO* 2050 (de Vries, personal communication). Both Suess and de Vries, however, considered that these dates were best regarded as minimum rather than absolute, owing to the well-known greatly increased disturbance of small contaminations, etc., on readings of this great age. On the whole, therefore, the projection of a line drawn from *GRO* 2586 through the midpoint between the readings for layers XX and XXI would seem to provide as reasonable a first approxi-

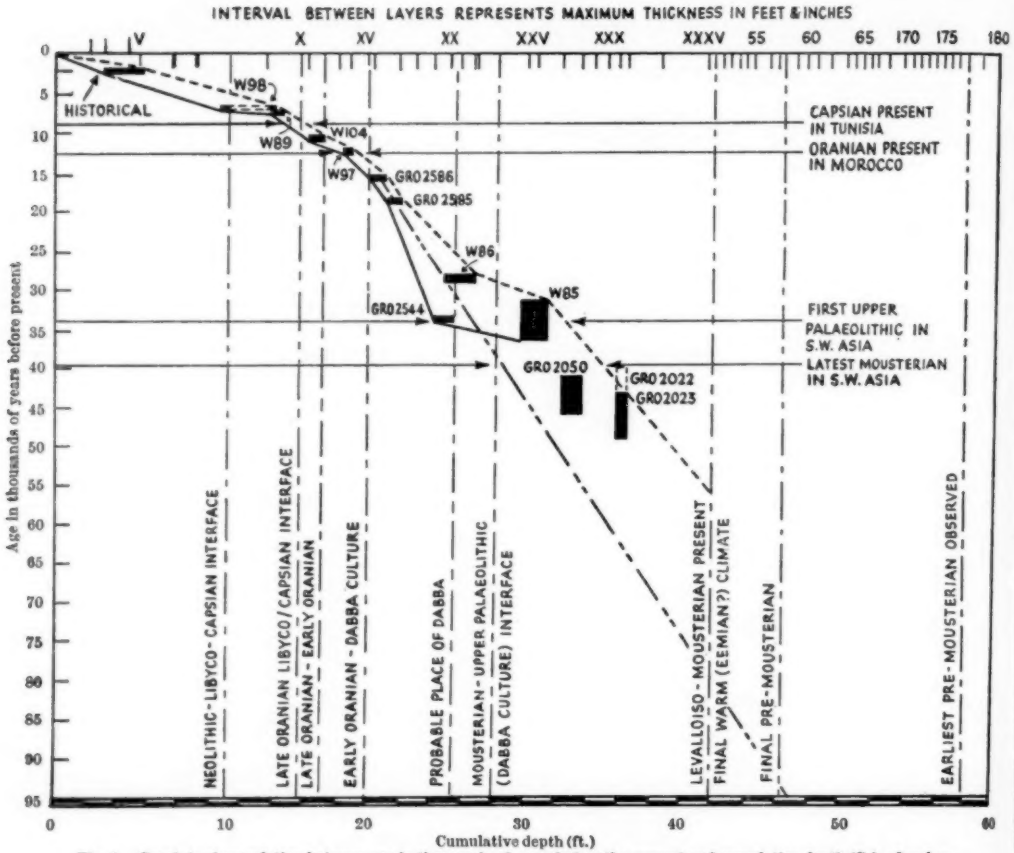


Fig. 1. Graph to show relation between age in thousands of years before the present and cumulative depth (ft.), of carbon samples from the Haua Fteah, Cyrenaica, Libya. The rectangles represent the depth through which the sample was taken and the standard error of the carbon-14 reading expressed in years. The central broken line represents the hypothetical trend beyond the range of the readings regarded as most reliable. The numbers at the top of the diagram are the reference numbers assigned to each layer

mation to the possible ages of the earlier interfaces as the present data allow. Although a wide margin must naturally be left to alternative estimates, an age for the earliest occupation horizon within the limits 100,000–150,000 seems probable.

It is interesting to set this against the geological evidence for an Eemian date recently established for the closest typological homologue, the Pre-Aurignacian of the Levant[7], since the end of the Eemian according to most carbon-14-based estimates would have occurred between 55,000 and 75,000 (refs. 8*a*, *b*, 13).

Using the same gradient, an age of 38,000 would be indicated for the Mousterian–Upper Palæolithic interface at the Haua, which would compare with previous estimates for the corresponding horizon at Shanidar (Kurdistan) of 35,000–40,000 (refs. 9, 10) $\geqslant$ 32,000 at Kara Kamar (Afghanistan) (ref. 10), and $>$ 32,000 (ref. 12) (estimated 36,000, ref. 13) at Istallöskö (Hungary). On the other hand, a date $<$ 39,500 (ref. 14) is indicated by the presence of a Mousterian horizon of this age at Tabun (Palestine).

Again, the age of 14,000 indicated for the earliest Oranian at the Haua may be compared to 12,000 recently established for the middle Oranian horizon at Taforalt (Morocco)[15], whence the earliest Oranian at this site can be estimated as between 11,000 and 15,000 on the stratigraphical context.

On the basis of these figures it may be said that the chronology suggested by the combined carbon-14 readings and stratigraphy at the Haua Fteah is in general agreement with available carbon-14 dates for the corresponding cultural events in not too distant areas.

C. B. M. McBurney
Department of Archæology and Anthropology,
University of Cambridge.

[1] McBurney, C. B. M., Trevor, J. C., and Wells, L. H., *Nature*, **172**, 889 (1953).
[2] McBurney, C. B. M., Trevor, J. C., and Wells, L. H., *J. Roy. Anthropol. Inst.*, **83**, 71 (1953).
[3a] McBurney, C. B. M., in *Neanderthal Centenary*, 253 (Utrecht, 1958); [3b] *The Stone Age of Northern Africa*, **5** (Harmondsworth, 1960).
[4] Rust, A., *Die Höhlenfunde von Jabrud* (Neumünster, 1950).
[5] Higgs, E. S., *Man* (1961).
[6] Suess, H. E., *Science*, **120**, 467 (1954).
[7] Garrod, D. A. E., *Bull. Musée, Beyrouth*, **16**, 7 (1961).
[8a] de Vries, Hl., *Eiszeitalter und Gegenwart*, **9**, 10 (1958); [8b] *Archæologia Austriaca*, **25**, 35 (1959).
[9] de Vries, Hl. (personal communication).
[10] Solecki, R. S., *Trans. N.Y. Acad. Sci.*, II, **21**, 712 (1959).
[11] Coon, C. S., *Science*, **123**, 447 (1956).
[12] Vertés, L., and de Vries, Hl., *Arch. Értesitö*, **86**, 195 (1959).
[13] Movius, H. L., *Current Anthropol.*, **1**, 355 (1960).
[14] de Vries, Hl. (personal communication).
[15] See Balout, L., in *Prehistoire de l'Afrique du Nord* (Paris, 1955).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 20

CIBA FOUNDATION (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 5 p.m.—Prof. E. Katchalski (Weizmann Institute of Science, Rehovoth): "Synthetic Macromolecules as Protein Models" (Thirteenth Ciba Foundation Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Electrical Accidents and their Causes, including the Legal Aspects", opened by Mr. S. J. Emerson, O.B.E.

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. H. Gysin: "Triazine Herbicides, their Chemistry, Biological Properties and Mode of Action".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. F. A. E. Pirani and Dr. G. F. Elliott: "Ballistic Missiles and Nuclear Weapons—Effects of Nuclear Weapons".* (Sixth of seven lectures in War Studies. Further lecture on November 27.)

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. T. Sorgenfrei (Copenhagen): "Tertiary of North-West Europe".* (Further lectures on November 21 and 23).

UNIVERSITY OF LONDON (in the Large Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.30 p.m.—Prof. C. R. Rao (Indian Statistical Institute, Calcutta): "Some New Tests in Large-Sample Theory".* (Further lectures on November 24 and 27.)

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Mechanical Handling of Small Parts with special reference to Automatic Assembling Equipment".

INSTITUTE OF METAL FINISHING (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. L. E. Probert and Mr. J. Rollinson: "Detection and Removal of Hydrogen Absorbed during Chemical and Electrochemical Processing".

Tuesday, November 21

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London W.C.2), at 5.30 p.m.—Discussion on "The Precision Required in the Calibration of Industrial Instruments", opened by Mr. P. M. Clifford and Mr. A. Felton.

PARLIAMENTARY AND SCIENTIFIC COMMITTEE (in Committee Room 12, House of Commons, London, S.W.1), at 5.30 p.m.—Meeting of the General Committee. Prof. P. B. Medawar, F.R.S.: "The Impact of Biological Research on Advances in Medical Science".

RESEARCH DEFENCE SOCIETY (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Sir Hugh Linstead: "On Taking Account of Public Opinion". (Thirteenth Stephen Paget Memorial Lecture).

SOCIETY FOR ANALYTICAL CHEMISTRY, PHYSICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Annual General Meeting followed by the Retiring Chairman's address.

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. A. R. Harrison: "Primary Hyperparathyroidism".* (Eleventh of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof K. R. Popper: "Science and the Growth of Knowledge".* (First of four Shearman Memorial Lectures. Further lectures on November 23, 28 and 30.)

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Prof. D. R. Cox: "The Role of Statistical Methods in Science and Technology"* (Inaugural Lecture).

Tuesday, November 21—Wednesday, November 22

INSTITUTION OF GAS ENGINEERS (at Church House, Westminster, London, S.W.1), at 9.30 a.m. on Tuesday and 9.15 a.m. on Wednesday—27th Autumn Research Meeting.

Wednesday, November 22

ZOOLOGICAL SOCIETY OF LONDON (NUFFIELD INSTITUTE OF COMPARATIVE MEDICINE) (at the Zoological Society, Regent's Park, London, N.W.1), at 10 a.m.—Conference on "Arterial Diseases".

GEOLOGICAL SOCIETY OF LONDON (at the Royal Institution, Albemarle Street, London, W.1), at 2 p.m.—Symposium on "Depth and Tectonics as Factors in Regional Metamorphism".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Prof. G. H. Lathe: "Bile-Pigment Metabolism".*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Miss Sylvia Crowe: "Landscape and Civilization".

INSTITUTE OF FUEL (at the Royal United Service Institution, Whitehall, London, S.W.1), at 5.30 p.m.—Mr. L. E. Reed and Mr. S. C. Wallin: "The Effect of Operating Conditions on the Performance of Different Types of Oil Burners".

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. A. S. Aldred: "Electronic Analogue Computer Simulation of Multi-Machine Power System Networks"; Mr. J. G. Miles: "Analysis of Overall Stability of Multi-Machine Power Systems".

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Adaptive Optimizing Control".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Prof. E. Giffen: "Reflections on the Institution's Role in Engineering Education" (James Clayton Lecture).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—"Food Technology in Norway". (The film and members' impressions of the 1961 tour.)

UNITED STATES INFORMATION SERVICE (in the American Embassy Theatre, American Embassy (Upper Brook Street entrance), London, W.1), at 6.30 p.m.—Three films from the series "Planet Earth": "Research by Rockets"; "Cosmic Rays" and "Radio Waves".*

BRITISH PSYCHOLOGICAL SOCIETY, MEDICAL SECTION (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 8.30 p.m.—Mr. Murray Jackson, Mr. Michael Fordham and Mr. Kenneth Lambert: "Jung's Later Work" (C. G. Jung Commemorative Meeting).

Thursday, November 23

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. J. H. Burns: "Jeremy Bentham and University College".*

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 2 p.m.—Meeting on "Industrial Cell Culture and Vaccine Production".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Mr. M. N. Parker and Mr. James Dawson: "Tug Propulsion Investigations".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. Keith Runcorn: "Palæomagnetism"; Prof. P. Allen: "Palæotemperatures".

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. B. R. Rabin: "Some Aspects of Enzyme Catalysis".* (Further lecture on November 30.)

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C.2), at 5 p.m.—Dr. George Taylor: "Britain's Botanical Power House".* (Further lecture on November 30).

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. F. C. McLean, Mr. H. W. Baker and Mr. C. H. Colborn: "The B.B.C. Television Centre and Its Technical Facilities".

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Prof. Hermann Bondi: "Ideas of Special Relativity".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. I. E. Bush: "Hormones of the Adrenal Cortex".* (Twelfth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. T. Sorgenfrei: "Tertiary of North-West Europe".* (Last of three Special University Lectures.)

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The 57 Effects of an Electrical Current" opened by Dr. G. S. Brosan.

UNIVERSITY OF LONDON (at Wye College, Wye, near Ashford, Kent), at 8.15 p.m.—Dr. H. L. Jensen (Denmark): "Recent Advances in Soil Microbiology".*

Friday, November 24

ASSOCIATION OF APPLIED BIOLOGISTS (joint meeting with the HORTICULTURAL EDUCATION ASSOCIATION, at the Royal Horticultural Society, Vincent Square, London, S.W.1), at 10.50 a.m.—Meeting on "Pests and Diseases of Ornamental Plants".

ROYAL ASTRONOMICAL SOCIETY (joint meeting with the ROYAL METEOROLOGICAL SOCIETY, at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "The Density and Composition of the Atmosphere Above 100 km.". Chairman: Mr. J. A. Ratcliffe, F.R.S.

UNIVERSITY OF LONDON (in the Manson Lecture Theatre, London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.30 p.m.—Prof. C. R. Rao (Indian Statistical Institute, Calcutta): "On Problems of Discrimination with Multiple Characters".* (Further lecture on November 27.)

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. R. Catch: "The Production of Organic Compounds Labelled with C-14 or Tritium".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. P. B. Hirsch: "Seeing Atomic Defects in Metals".

Friday, November 24—Saturday, November 25

Botanical Society of the British Isles (at the Linnean Society, Burlington House, Piccadilly, London, W.1). Conference on Local Floras and Annual Exhibition Meeting.

Saturday, November 25

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. C. M. Mitchell: "The Folk Life of the English People".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer (preferably with an interest in theoretical or experimental plasma physics) in Physics—The Secretary, The Queen's University, Belfast, Northern Ireland (November 24).

Junior Lecturer (preferably with a registrable medical qualification) in Bacteriology—The Registrar, Trinity College, Dublin 2 (November 27).

Physics Master (graduate with a good honours degree)—The Head Master, City of Oxford High School, Oxford (November 30).

Research Fellow (with a postgraduate degree in geophysics, physics or geology, and preferably, though not necessarily, previous experience in seismological work) in Seismology—The Secretary, The University, 38 North Bailey, Durham (December 1).

Assistant Biochemist (with a degree or equivalent qualification in chemistry, with experience in, or wishing to take up, clinical biochemistry)—The Secretary, Welsh National School of Medicine (University of Wales), 34 Newport Road, Cardiff (December 2).

Lecturers in Pure Mathematics and in Applied Mathematics—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (December 4).

Lecturer (with postgraduate experience in research coupled with a keen interest in teaching) in Physics at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, December 4).

Technical Officer (with a recognized degree or National Diploma in Agriculture, and preferably experience in seed production) for Seed Multiplication—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (December 4).

Burnett-Fletcher Chair of History and Archæology—The Secretary, The University, Aberdeen (December 9).

Lecturer (with an honours degree in electrical or electronic engineering) in the Department of Electrical Engineering—The Registrar, The University, Leeds (December 14).

Lecturer (with an honours degree in psychology) in Psychology at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 15).

Lecturer in Psychology at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (December 20).

Lecturer (preferably with qualifications in one or more of the following fields: geomorphology, biogeography, and the geography of soils) in Geography at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, December 30).

Reader in Physics—The Registrar, The University, Hull (December 31).

Mary Glendining Chair of Medicine in the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 15).

Chair of Veterinary Science in Massey Agricultural College (University of New Zealand), Palmerston North—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (February 1).

Computer Programmer in the Nuclear Physics Research Laboratories, to work with a research group studying high energy nuclear physics—The Registrar, The University, Liverpool, quoting Ref. No. CV/N/543.

Lecturer (well qualified, and preferably with industrial and teaching experience) in Mathematics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 32/G.

Lecturer (with a first degree or equivalent qualification in chemical engineering, chemistry, mathematics or physics, and preferably some industrial experience) in Chemical Engineering—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 31/G.

Master to teach Physics throughout the School, with Sixth Form and University Scholarship work—The Headmaster, King's College School, Wimbledon, London, S.W.19.

Postdoctoral Fellow and a Postgraduate Student in Chemistry, for work on the fluorescence of aromatic hydrocarbons under the direction of Dr. B. Stevens—The Registrar, The University, Sheffield.

Professor of Thermodynamics at the University of Malaya, Kuala Lumpur, to develop research in applied thermodynamics and to supervise courses already in existence—Department of Technical Co-operation, 26 King Street, London, S.W.1, quoting MAL/861/TCS.

Scientific Officer or Senior Scientific Officer (honours graduate in chemistry or biochemistry with interests in the isolation and chemistry of proteins and in studies of enzymes) in the Physiology Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, near Reading, quoting Ref. 61/16.

Senior Lecturer in Mathematics; and a Research Assistant in Mathematics—The Registrar (Department D), Bradford Institute of Technology, Bradford 7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research. Forest Products Research. Bulletin No. 46 : Identification of Hardwoods : a Microscope Key. By J. D. Brazier and G. L. Franklin. Pp. viii + 96 + 32 plates. (London : H.M. Stationery Office, 1961.) 10s. net. [258

Fitzwilliam Museum, Cambridge. Annual Report for the year ending 31 December 1960. Pp. 8 + 4 plates. Friends of the Fitzwilliam Museum Fifty-second Annual Report for the year 1960. Pp. 8. (Cambridge : The Fitzwilliam Museum, 1961.) [258

Ministry of Housing and Local Government. Essex Rivers and Stour Hydrological Survey. Hydrometric Areas 36 and 37. Pp. iv + 122. (London : H.M. Stationery Office, 1961.) 20s. net. [258

Iron and Steel Institute. Bibliographical Series, No. 15b : The Rolling of Iron and Steel (1955–1960). Pp. 164. (London : The Iron and Steel Institute, 1961.) 40s. [258

Philosophical Transactions of the Royal Society of London. Series A : Mathematical and Physical Sciences. No. 1036, Vol. 254 (24 August 1961) : Axially Symmetric Plastic Deformations in Soils. By A. D. Cox, G. Eason and H. G. Hopkins. Pp. 1–45. (London : Royal Society, 1961.) 14s. [258

Gateways : Guide to Courses of Further Education in Middlesex, 1961–1962. Part 2 : General Interest and Recreational Courses. Pp. 52 + 4 plates. (London : Middlesex County Council Education Committee, 1961.) [258

N.L.L. News, No. 1 (August, 1961). (A News-Sheet of the National Lending Library for Science and Technology.) Pp. 4. (Boston Spa : National Lending Library for Science and Technology, D.S.I.R., 1961.) [258

Report of the Hydrographer of the Navy for the year 1960. Pp. 33 (4 plates). (London : Admiralty, 1961.) [258

Other Countries

Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin. Klasse für Medizin. Jahrgang 1960, Nr. 1: Chemische Mutagenese-Erwin-Bauer-Gedächtnisvorlesungen I, 1959: Veranstaltet vom Institut für Kulturpflanzenforschung Gatersleben der Deutschen Akademie der Wissenschaften zu Berlin, 26–28, Juli, 1959. Herausgegeben von H. Stubbe. Redaktion: H. Bohme. Pp. vi + 136 + 3 tafeln. (Berlin: Akademie-Verlag, 1960.) 18.50 D.M. [258

Deutscher Wetterdienst. Deutsches Meteorologisches Jahrbuch, Saarland, 1945–1955. Pp. 38. (Offenbach a. M.: Selbstverlag des Deutschen Wetterdienstes, 1961.) [258

Dental Caries from the Nutritional Point of View. By Alfred Åslander. Pp. 7. (Stockholm: Alfred Åslander, Royal Institute of Technology, 1961.) [258

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 76: Spores with Proximal Radial Pattern from the Devonian of Canada. By D. C. McGregor. Pp. ix + 11 + 1 plate. (Ottawa: Queen's Printer, 1961.) 75 cents. [258

Geological Maps of Canada. Map 1092A: Sevogle, Northumberland and Gloucester Counties, New Brunswick. (Sheet 21 P/4 West Half). Map 2–1961: Rabbit River, British Columbia. (Sheet 94M). Map 5–1961: Dean Lake, Ontario. (Sheet 41 J/3.) Map 6–1961: Wakwekobi Lake, Ontario. (Sheet 41 J/6.) (Ottawa: Geological Survey of Canada, 1961.) [258

Forty-fourth Annual Report of the National Research Council of Canada, 1960–61, including the Annual Reports of the Medical Research Council and Canadian Patents and Development Limited. Pp. 48. (N.R.C. 6322.) (Ottawa: Queen's Printer, 1961.) [258

Canada. Division of Building Research: National Research Council. Canadian Building Digest No. 20: Corrosion in Buildings. By P. J. Sereda. Pp. 4. (Ottawa: Division of Building Research, National Research Council, 1961.) [258

VIe Symposium International de Nematologie, Gand, Belgique, 24–28 juillet, 1961—Résumés des Communications. Pp. 122. (Bruxelles: Ministère de l'Education Nationale et de la Culture et du Ministère de l'Agriculture, 1961.) [258

Canada. Department of Northern Affairs and National Resources. National Museum of Canada. Bulletin No. 174 (Anthropological Series, No. 51): Tsimsyan Myths. By Marius Barbeau. Pp. v + 97. 1 dollar. Natural History Papers. No. 11 (July 24, 1961): Placement of the Prowfishes, *Zaproridae*, in the Subfamily Stichaeoidae. By D. E. McAllister and R. J. Krejsa. Pp. 4. No. 12 (July 3, 1961): New Records of Fresh-Water Amphipod Crustaceans from Oregon. By K. L. Bousfield. Pp. 7. (Ottawa: Queen's Printer, 1961.) [258

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.
ST. MARTIN'S STREET, LONDON, W.C.2
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2.
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

XUM